Naval Medical Research Unit – Dayton

Technical Report

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Health Risk Assessment of Women in Submarines: Reproductive and Developmental Toxicity Evaluation of Major Submarine Atmosphere Components (CO, CO_2, and O_2) in Rats (*Rattus norvegicus*) – Phase II (Neurological and Reproductive Performance Study)

DANIEL J. HARDT, R. ARDEN JAMES, CHESTER P. GUT, Jr., SHAWN M. MCINTURF, and MICHAEL L. GARGAS

Approved and Released by:
CAPT K. A. Syring, MSC,
USN Commanding Officer

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Abstract

This study evaluates general, reproductive and developmental effects on male and female rats exposed to mixed atmospheres of three critical submarine air components (CO, CO_2, and O_2) at concentrations representing the submarine standards for continuous exposure limits (CELs) and emergency exposure limits (24-hour and 1-hour EELs). The complete study is divided into three phases designed to determine whether the existing standards for these gases, in combinations, are health protective for both male and female submarine crew members. Phase 1 was a range finding study to screen for overt toxicities after 14 days of exposure. Phase 2, described in this report, provides a 28-day exposure evaluation of neurological and reproductive performance, in addition to general toxicities. Finally, Phase 3 will provide a 90-day sub-chronic, two generation, developmental and reproductive study that will also evaluate the reproductive ability of offspring exposed *in utero* to gestation day 19. This technical report presents the findings of Phase 2 of the study, which evaluated four groups of 28 male and 28 female rats exposed via whole body inhalation to clean air (0.4 ppm CO, 0.1% CO_2, 20.4% O_2), a low-dose gas mixture (5.0 ppm CO, 0.44% CO_2, 16.9% O_2), a mid-dose gas mixture (13.7 ppm CO, 1.6% CO_2, 15.9% O_2), and a high-dose gas mixture (85.6 ppm CO, 2.4% CO_2, 14.9% O_2) for 23 hours per day for 28 days. Following exposure, the rats bred to produce offspring, with parents and offspring examined for neurobehavioral and physiological effects. No adverse reproductive effects were observed in exposed parents during mating, gestation or parturition. No adverse health effects were identified in exposed parents or offspring based on clinical chemistry, organ and whole body weight changes, or histopathology. No developmental or functional deficits were observed in exposed parents or offspring related to motor activities, exploratory behavior or higher-level cognitive functions (learning and memory). Only minimal effects were discovered in the parent-offspring emotionality tests. Significant increases in hematopoietic parameters were observed in offspring of exposed parents compared to controls; however, the increased values remained within, or close to, the normal clinical ranges for blood cells and components. The potential

mechanism(s) of action for this effect will be evaluated further in Phase 3. In conclusion, 28-day exposures to the elevated concentrations of the submarine atmosphere gases described did not affect the ability of rats to reproduce and did not result in any significant developmental deficits in their offspring.

Keywords: Inhalation, carbon monoxide, carbon dioxide, hypoxia, reproductive toxicity

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Introduction

Submarine atmospheres present a unique and closed occupational environment, with personnel being sub-chronically exposed to low-level concentrations of chemicals and chemical mixtures for 24 hours per day. Congress has recently passed legislation that will allow women to serve aboard submarines; therefore, it is imperative to re-evaluate the current submarine breathing air standards, such as emergency exposure levels (EELs) and continuous exposure levels (CELs), with a special focus upon potential reproductive and developmental effects, as well as sex-specific effects. Based on previous efforts (National Research Council 2007, 2008, 2009), the atmospheric components of carbon monoxide (CO), carbon dioxide (CO_2), and oxygen (O_2) are considered among the highest health concerns for submarine atmospheres.

Several studies have examined the developmental and reproductive effects of hypoxia, and the inhalation of elevated CO or CO_2 concentrations. Epidemiological evidence attributes several developmental and reproductive effects in humans to hypoxia and CO exposure (Bass et al., 2004; Salam et al., 2005). Published studies in hypoxic animals have indicated decreases in mating rates, sperm production and litter sizes at exposures of 12% O_2 (Cikutovic et al., 2009) and neurobehavioral deficits at exposures of 9.5% O_2 (Chahbourne et al., 2009; Dubrovskaya and Zhravin, 2010). Published findings implicate high CO_2 inhalation exposures with reversible degenerative changes in the testes of rats at 2.5% CO_2 (Vandemark et al., 1972); decreased sperm production in mice at 35% CO_2 (Mukherjee and Singh, 1967); decreased fetal viability and increased heart malformations in rats at 6% CO_2 (Haring, 1960); and, neuro-behavioral deficits in rats at 7% CO_2 (De la Fuente et al., 2003). In addition, published findings implicate high CO inhalation exposures with decreased fetal weight and viability in mice at 125 ppm (Singh and Scott, 1984), rats at 125 ppm (Prigge and Hochrainer, 1977; Carmines et al., 2007), and rabbits at 90 ppm (Astrup et al., 1972); placental hypertrophy in rats at 100 ppm (Lynch and Bruce, 1989), heart hypertrophy at 60 ppm (Prigge and Hochrainer, 1977; Barbe et al., 1999);

decreased hematopoiesis at 250 ppm in rats (Prigge and Hochrainer, 1977); decreased splenic macrophage function in rats at 75 ppm (Giustino et al., 1993); skeletal malformations in mice at 250 ppm (Schwetz et al., 1979); and, neurobehavioral deficits in mice at 125 ppm (Singh, 1986) and rats at 75-150 ppm (Di Giovanni et al., 1995; De Salvia et al, 1995). However, there are no data that assess the combined effects of these gases as mixtures, nor that assess the adverse health effects of these gases after prolonged, continuous (24 hour per day) exposures.

Assessing the health risk to female crew members in submarines is a complex and controversial issue (Kane and Horn, 2001). This research is being conducted to clarify the potential impacts of these mixed gases on male and female reproductive and developmental health, as well as the overall mission effectiveness of the submarine community. When adequate human data are lacking, the primary alternate method for establishing the health risk from a chemical substance is to perform toxicity studies in animals, and then use the research principles that have been proven to be predictive, robust, and valid for extrapolating the animal results to humans.

The purpose of this study is to evaluate the general, reproductive and developmental toxicity in male and female rats exposed for 28 days via whole body inhalation to various combinations of the three major submarine atmospheric components (increased CO and CO_2, and decreased O_2), and to use these data to guide the 90-day sub-chronic study (Phase 3).

The O_2, CO, and CO_2 exposure concentrations were selected based upon existing standard limits promulgated within the *Technical Manual for Nuclear Powered Submarine Atmosphere Control* (NAVSEA S9510-AB-ATM-010 REV 2). The low-dose group concentrations are based on the 90-day CEL (average onboard levels); the mid-dose group concentrations are based on the 24-hour CEL (maximum onboard levels); and, the high-dose group concentrations are based on the 1-hour EEL (emergency levels).

Experimental Design

The study will be performed in three consecutive phases. Phase 1 was a range finding study described in a previous report (Hardt et al., 2011) and involved continuous exposures to male and female rats for 14 days to the test atmospheres with toxicity assessments performed on vital organs and reproductive tissues. This Phase 2 report describes male and female rats exposed for 28 days with neurological and reproductive performance assessed, in addition to general toxicity. The first generation of rats (F1) from Phase 2 were also assessed for general health conditions and gross malformations, but were not exposed to the test atmospheres. The 90-day, two generation sub-chronic study (Phase 3) will be modeled after the USEPA guidelines for assessing "*Reproduction and Fertility Effects*" (OPPTS 870.3800). The male and female rats will be exposed to the same three test atmospheres for a continuous 90 day period that includes gestation, and will be assessed for toxicity, as well as neurological and reproductive effects. Phase 3 (F1) offspring will not be exposed *in utero* up to gestation day 19, and will be evaluated for general toxicity, malformations, and neurological and reproductive abilities. F2 offspring resulting from the mating of F1 rats will not be exposed, but will be evaluated for general toxicity and gross malformations to assess any delayed developmental effects or toxicity, and to assess the reproductive capability of the F1 generation. This current report addresses only Phase 2.

Materials and Methods

Animal Exposure

Four groups of animals (submarine atmosphere target concentrations) were exposed to clean air (0.4 ppm CO, 0.1% CO_2, 20.4% O_2), a low-dose gas mixture (5.0 ppm CO, 0.44% CO_2, 16.9% O_2), a mid-dose gas mixture (13.7 ppm CO, 1.6% CO_2, 15.9% O_2) and a high-dose gas mixture (85.6 ppm CO, 2.4% CO_2, 14.9% O_2) for 23 hours per day for 28 consecutive days. Each exposure group was stagger-started by one day to minimize disturbance of the animals and maximize resources during loading and unloading operations.

Animals

A total of 224 CD® IGS rats, 51-54 days-old, were purchased from Charles River Laboratories (Wilmington, MA). The rats were randomly divided into four groups of 28 males and 28 females. The rats were provided husbandry conditions consistent with practices recommended by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), and in compliance with the National Research Council's "Guide for the Care and Use of Laboratory Animals" (ISBN-10:0-309-15400-6). After arrival at the facility, the rats underwent a two week quarantine period in the animal vivarium, which included four days to acclimate to the exposure cage units (cage training). Rats were placed in stainless steel cage units for increasing periods of time (2, 4, 6 and 8 hours) on four consecutive days during the week prior to the study start, and were returned to polycarbonate cages between training periods. Following acclimatization, the rats were placed in the cage units for the duration of the inhalation study except when the cages were changed (weekly), or when rats were weighed (weekly) and monitored for estrous cycle alterations via vaginal lavage and cytology assessment. Rats were provided food and water *ad libitum* throughout the experiment, and were kept on a 12 hour light/dark cycle.

Chemicals

Rats were exposed to clean air or atmospheres with reduced O_2 and increased CO and CO_2. Clean air for the control and exposure system was from an air circulating system using a turbine blower (The Spencer Turbine Co., Windsor, CT) with a room air intake filtered through a high-efficiency particulate air (HEPA) filter to replace the air. One cylinder (4.25 cubic meters) of CO (99.999%) and five cylinders (180 L dewars or 414 pounds liquid CO_2 per cylinder) of CO_2 (99.99%) were purchased from AirGas, Lansing, MI. The O_2 concentrations were reduced to test conditions by dilution with appropriate amounts of nitrogen (N_2) provided from a nitrogen generator (Parker Balston Model DB-5, Summit Industries, Inc., Dayton, OH). The nitrogen generator produced 95 to 99% N_2 from in-house compressed air filtered for water and oils.

Inhalation Exposure Chambers

Rats were exposed in a one cubic meter whole body exposure chamber (1 m^3, H1000, Lab Products, Seaford, DE). One chamber was used for each concentration including a control chamber. Two stainless steel cage units (R-32, Lab Products, Seaford, DE) were used to contain the animals during inhalation exposures and served as the domiciliary housing during periods of non-exposure. Each R-32 cage unit housed 32 rats, and one cage unit was placed in the middle and one in the lower section of each 1 m^3 chamber. The dimensions for each rat compartment within the R-32 cage units were 14.0 x 14.5 x 20.3 centimeters (W x L x H) and provided 203 square centimeters of floor space. Cage units were changed weekly for the duration of the inhalation exposures. Stainless steel pans were changed daily and placed under each set of stainless steel cages to collect the urine and feces.

Inhalation Exposure Chamber Operation

The inhalation exposure chambers were operated as a push-pull system. Air was pushed into the inlet of the chambers from an air circulating system using a turbine blower (The Spencer Turbine Co., Windsor, CT) with a room air intake to replace used air through a high-efficiency particulate air (HEPA) filter. Air was pulled from the exhaust of the chambers through a manifold using an exhaust fan on the roof of the facility. The target inlet air flow rate in the mixed atmosphere chambers was set to 200 to 250 L/min, providing approximately 12 to 15 air changes per hour. Inlet air flows were a sum total of the clean air, CO flow, CO_2 flow and N_2 flow. Inlet air flows were controlled by a manually operated gate valve. Inlet air flows were monitored by mass flow monitor (Model HFM-200 LFE, Teledyne-Hastings Instruments, Pittsburgh, PA) connected to a laminar flow element (Model HFM-200 LFE, Teledyne-Hastings Instruments, Pittsburgh, PA). Each of the mass flow monitors was connected to a four-channel power supply (Model THPS-400-115, Teledyne-Hastings Instruments, Pittsburgh, PA).

The inlet air flow for the control chamber was set to a target flow rate of approximately 390 L/min (approximately 23.5 air changes per hour) to dilute the CO_2 concentrations produced by the collective exhaled breath from the animal load. The higher flow rate to the control chamber resulted in somewhat lower humidity conditions for rats in the control group in comparison to the rats from the dose groups, but this difference is not expected to affect study results.

The chamber exhaust flow for the mixed atmosphere exposure chambers was adjusted with a manually operated gate valve to maintain a slight negative pressure relative to the room during the exposure to prevent the test atmosphere from entering the laboratory area in the event of leaks. The control chamber exhaust flow was adjusted to maintain a slight negative pressure relative to the room and the control chamber door latches were left open to allow room air to enter thereby diluting the CO_2 produced by the exhaled breath of the animal load to the lowest concentrations possible.

The static pressure of each inhalation chamber was determined using both a magnehelic gauge (Model 2304, Dwyer Instrument Co., Michigan City, IN) with a large visual display and an electronic sensor (Model ZPS-05-SR09-EZ-ST-D, Building Automation Products, Inc., Gays Mills, WI).

Temperature and Humidity

Temperature and relative humidity were measured by a temperature and relative humidity probe (Model HF532WB6XD1XX, Model HC2-S, Rotronics Instruments, Inc., Hauppauge, NY) located inside of each exposure chamber. The target temperature was maintained between 64 to 79 °F and the target relative humidity was between 30 and 70%.

Atmosphere Generation

All test chemical gases for the mixed atmospheres were regulated by mass flow meters (Model HFC-202, Teledyne-Hastings Instruments, Pittsburgh, PA) at flow rates appropriate to maintain target concentrations of mixed atmospheres for each of the target doses. Each of the mass flow meters were connected to a four-channel power supply (Model THPS-400-115, Teledyne-Hastings Instruments, Pittsburgh PA) and manually adjusted to the appropriate channel of a four channel power supply. Figure 1 shows a diagrammatic representation of the exposure system.

Test Atmosphere Monitoring

The mixed test atmosphere of each of the four inhalation chambers was monitored continuously with a multiple gas analyzer (Model VA-3113, Horiba Instruments, Inc. Moon Township, PA). Each instrument contained a magneto-pneumatic (MP) sensor for O_2 measurements and two non-dispersive infrared analyzers (NDIR) for CO and CO_2 measurements. The oxygen sensor on the gas analyzer used for the high concentration chamber was damaged by moisture in the sample line and failed on Day 15. The analyzer was switched with the control chamber analyzer. A separate oxygen analyzer (Model CO6689-B1, Teledyne Analytical Instrument, City of Industry, CA) was then used to monitor the oxygen levels in the control chamber. Each instrument was calibrated using a N_2 dilution manifold and varying amounts of calibration gases (Airgas, Dayton OH): 500 ppm CO in N_2 for the CO NDIR, 5% CO_2 in N_2 for the CO_2 NDIR, and room air (20.9% O_2) for the O_2 MP and microfuel cell. Each instrument was zeroed using N_2.

Automated Alarm System

The monitoring sensors for the key parameters of temperature, relative humidity, airflow, CO concentration, CO_2 concentration and O_2 concentration within the inhalation chambers were electronically connected to an alarm system (Model FGD-2000, Sensaphones, Phonetics, Inc., Aston, PA) that automatically contacted the technician on duty if the electronic signal fell outside

of the acceptable range, ensuring prompt correction. This system recorded data every 30 seconds and served to back-up data in the event that the primary data recording system failed.

Exposure Data Collection

Data were automatically collected by computer using LabView software (v.10.0, National Instruments, Austin, TX). Data were collected every 10 seconds for temperature, humidity, supply air flow, CO concentration, CO_2 concentration, O_2 concentration and static pressure for all groups. In addition, for the low, mid and high dose groups, data were also collected every 10 seconds for CO flow rate, CO_2 flow rate and N_2 flow rate. The 24-hour daily data for each dose group were collected from approximately 0900 until 0900 the following day. Periods when the chambers were opened for animal husbandry and animal procedures or power failures due to significant weather were included in the daily averages to reflect the actual average exposure concentrations experienced by the rats. Data were eliminated from the daily average for equipment malfunctions if it was determined that the data were not representative of chamber conditions (e.g., excess humidity in sample line or oxygen sensor failure). At the end of each day, the average, standard deviation, minimum values, maximum value and the total number of data values were calculated. Daily averages were used to calculate the average of daily averages, standard deviation of daily averages, minimum daily average, maximum daily average and number of daily averages.

Study Day

A study day was defined as a 24-hour period generally from approximately 0900 until 0900 the following day. The study days were numbered consecutively from 1 to 31 corresponding to the first day when the control group was loaded into the control chamber until the last day when the final exposure group was removed from the high-dose chamber and reflected the staggered schedule for initiating exposures for the four groups. Exposures were interrupted each day for

approximately 15-60 minutes to remove urine and feces; inspect or change equipment; observe rats for health and well being; measure weights; and, record estrous cycle phase changes. Biological parameters measured for both the P1 (parents) and F1 (offspring) generations during exposure and post-exposure are listed in Table 1.

Mating and Monitoring of Pregnancy and Offspring

P1 Generation

Adult dams (parents of F1) were sorted into one of eight mating groups based on treatment group 24 hours post-exposure. The rats were randomly sorted into mating pairs with adult males who had either undergone the same exposure, or adult males who had no exposure (Table 2).

Male/female pairs were placed in open metal grid mating cages for 7 days. The bottoms of the mating cages were checked daily by study personnel for evidence of mating (e.g., seminal plugs). The date on which the seminal plug was discovered was designated Gestation Day 1 (GD1). When a seminal plug was discovered, the pair was removed from the mating cages, and placed in separate cages. If no evidence of mating was found for the mated pair by day 7, the male was returned to its home cage and the mating was recorded as a "failure". P1 females that did not produce evidence of a vaginal plug were weighed every 3 days for two weeks and monitored for significant weight changes, which were also evidence of a successful pregnancy, resorption, or a non-successful mating. The ultimate test of successful mating was the birth of a litter, since several dams gave birth to litters when no evidence of seminal plugs was discovered. To minimize handling of pregnant dams no weights were collected from GD14 to parturition. All dams not giving birth by day GD24 from the last possible date of mating were designated as non-breeders and were necropsied, with their organs sent for histopathological examination.

Beginning on GD19, P1 dams were monitored twice daily by study personnel for evidence of birth. Post natal day "zero" (PND0) was designated as the date birthing was discovered, or the date on which a complete delivery was discovered. Upon complete delivery, litter size (number of pups, living and stillborn), sex distribution, and litter pup weight for male and female groups, were recorded no later than PND1. The general physical condition of the litter (dam and pups) and number of malformations per litter (pups only) were also assessed twice daily by study personnel from PND1-4 and at least daily thereafter. No attempts were made to augment or supplement maternal care at any time during the study. All deceased pups were examined for gross defects, and necropsied for signs of neglect or an obvious cause of death. On PND4, F1 litters were standardized to 8 pups in as close to an equal male and female ratio as possible.

Randomly selected P1 animals underwent neurobehavioral assessments after mating was completed (32 males), or after weaning of the F1 pups (32 females).

F1 Generation

Eight litters (4 male pups, 4 female pups) from each mating group underwent neurobehavioral assessments from PND3 to PND8. P1 females were allowed to nurse and care for the selected F1 litters through PND20. All F1 offspring were weighed and weaned on PND21. After weaning, F1 offspring were randomly sorted by sex into groups of 2 – 4 animals of the same litter and housed until needed for neurobehavioral assessments, necropsy, maturation and euthanization. F1 offspring chosen for neurobehavioral assessments remained group housed until PND40, at which point they were moved to single housing to reach maturity (>PND51). After allowing offspring to reach sexual maturity (>PND51), adult neurobehavioral assessments were performed, followed by euthanized. Necropsies were performed on F1 offspring following weaning and after sexual maturity.

Special Breeding Group

Phase 3 of this study is to conduct mating and parturition during the exposure period. To discover any potential effects or interferences that the exposure system itself might have on mating success, 12 adult male and 12 adult female rats were placed singly into cages within an exposure chamber under the control (clean air) atmosphere. The rats were monitored daily by study personnel for any signs of injury or stress. On day 15 the animals were randomly sorted into mating pairs in the exposure cages and returned to the exposure chamber. Cage pans were monitored daily for evidence of mating (e.g. seminal plugs). The appearance of a seminal plug was considered evidence of a mating success. The date on which the seminal plug was discovered was designated GD1. When a seminal plug was discovered, the male was removed from the cage and euthanized. If no evidence of mating was found for the mated pair by day 7, the male was returned to its home cage and the mating recorded as a "failure". The inseminated females remained in the exposure cages and chambers until delivering of pups. The exposure cages were modified to have a solid bottom and bedding to prevent hypothermia in the pups, and any new born pups falling through the cages into the waste trays. Beginning on GD19, P1 dams were monitored twice daily by study personnel for evidence of birth. PND0 was designated as the date birthing was discovered, or the date on which a complete delivery was discovered. Upon complete delivery, the total number of pups (living and stillborn), sex distribution, litter pup weights for male and female groups, the general physical condition of the litter (dam and pups) and number of malformations per litter (pups only) were recorded no later than PND1. During their time in the exposure chamber the rats had access to food and water *ad libitum*, and were kept on 12 hour light and dark cycle. All dams and litters we euthanized when the last litter was delivered.

Estrous Cycle Monitoring

Estrous cycle phases were categorized for randomly selected female rats (8 per group) by the employment of vaginal lavage methods previously published by Marcondes et al. (2002). Dose group comparisons to controls were based on the proportion of days that rats were observed in each of the estrous cycle phases on 5-8 days during the 11-day estrus observation period. The metestrus and diestrus phases were combined into a single category. The evaluation period began following a full estrous cycle under exposure conditions. If the categorization was ambiguous (e.g., designated as positive for both the proestrus and the estrus phases), then each phase category was scored as an observation of 0.5 rat-days. If an insufficient number of cells were recovered to categorize an estrous cycle phase, then the data were excluded; as a result, the total number of rat-days sometimes varied between groups. The proportional differences between the dose groups and the controls were evaluated for statistical significance ($\alpha = 0.05$) for each of the estrous cycle phases.

Necropsy

On the day of the necropsy, male and female animals were anesthetized by CO_2 overdose until unresponsive, and then blood was sampled via cardiac puncture. After blood collection, the rats were decapitated and all target organs were harvested for analysis. Blood/serum was collected and processed for clinical chemistry and hematology analyses following standard laboratory procedures. Target tissues were harvested using standard necropsy methods. All blood and organ tissue samples were frozen at - 80^{o}C until processed for analysis.

Hematology

Complete blood count (CBC) analysis was performed on 40 µL samples of whole blood taken from each animal using a HemaVet® HV950 Blood Analyzer (Drew Scientific, Inc., Waterbury, CT). Parameters measured were: number per µL of white blood cells (WBC), red blood cells

(RBC), lymphocytes (LY), monocytes (MO), and granulocytes (neutrophils (NE); eosinophils (EO); basophils (BA)), % LY, % MO, % NE, % EO, % BA, grams hemoglobin (HB) per dL, % hematocrit (HCT), mean corpuscle volume (MCV), mean corpuscular hemoglobin (MCH) per pg, mean corpuscular hemoglobin concentration (MCHC) per dL, red blood cell distribution width (RDW), and number per µL of platelets (PLT).

Serum Chemistry

Serum chemistries were measured using a VetTest® 8008 Chemistry Analyzer (IDEXX Labs, Inc., Westbrooke, ME) and VetLyte® Electrolyte Analyzer (IDEXX Labs, Inc., Westbrooke, ME). A 100 µL sample of serum from each animal was analyzed for total protein (TP), albumin (ALB), alkaline phosphatase (ALKP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), cholesterol (CHOL), creatinine kinase (CK), creatinine (CREA), globulin (GLOB), glucose (GLU), total bilirubin (TBIL), triglycerides (TRIG), and major electrolyte concentrations (Na^+; K^+; Cl^-).

Tissue Histopathology

Select tissues and organs were fixed in formalin, properly sealed and packaged, and express shipped to the Seventh Wave Histology Laboratory, 743 Spirit 40 Park Drive, Chesterfield, MO, for histopathological analysis. The following tissues were prepared/submitted for evaluation: brain (basal ganglia, hippocampus and hypothalamus), heart, pancreas, liver, spleen, kidneys, adrenal glands, pituitary gland, male reproductive organs (testes, seminal vesicles and prostate), and female reproductive organs (ovaries, uterus, cervix and vagina). Permanent, formalin fixed, paraffin embedded, 5 micron, hematoxylin and eosin stained sections of select tissues are archived at NAMRU-D. Gross pathology was performed by LTC Deidre Stoffregen (VC, USA, DACVP), NAMRU-Dayton, WPAFB, OH.

Neurobehavioral Assessment

Following 28 days of submarine atmosphere exposures and subsequent home cage mating, neurobehavioral assessments were conducted for a random sample of the parental generation males and females (eight from each of the four exposure groups, respectively) and a subset of their offspring (Table 3). Females mated with exposed males and their litters (32) and females mated with unexposed males and their litters (32) were tested for early developmental deficits and maternal responses. On PND4, litters were culled down to eight randomly selected pups (4 males and 4 females) to be used for developmental tests. The pups were inspected for physical birth defects and non-selected pups were euthanized via CO_2 overdose. Following weaning on PND19-23, one male and one female from each paired-exposure group litter (64) were retained until sexual maturity to conduct further adult testing. Testing procedures followed developmental studies previously performed in this lab (McInturf et al., 2008; Arfsten et al., 2009). Pups were tested for righting reflex (Pellis et al., 1991) and separation distress (Bekkedal et al., 1999). Female parents were tested for maternal retrieval response (Hahn and Lavooy, 2005). Parent males and females and adult offspring were tested for motor activity (MA), and water-maze navigation using a modified Morris water-maze methodology (Morris, 1984; Buccafusco, 2001).

Assessments in Pups

Righting Reflex

Development of early motor coordination was assessed in the pups with the test of righting reflex on PND4 or PND5. Individual pups were placed in a supine position on a Plexiglas platform. The pups were gently held down by positioning an index finger along the abdomen. The finger was removed and the latency for the pup to roll over and obtain the prone posture with all four paws on the platform was timed. The procedure was immediately repeated two more times, for a total of three consecutive tests, and the scores were averaged for statistical analyses. If a pup failed to right within 60 seconds, it was classified as “timed out.”

Separation Distress

Emotionality was measured in the pups by recording the ultrasonic distress vocalizations (USV) emitted upon separation from the dam and littermates on PND7 or PND8. Tested pups were individually taken to a room separate from the home cages and placed in a glass jar containing bedding from the pups' home cage. A Petterson Ultrasound Detector (D240) set for 38-40 kHz using heterodyning was attached with audio cables to a Tascam (Da-20 MKII) digital audio tape recorder. The USV detector was hung 15 cm above the center of the jar. The total number of ultrasonic vocalizations was recorded for 60 seconds. Following all data collection, a trained technician replayed all recordings using the same Tascam digital audio tape recorder to score the number of vocalizations occurring during 60 seconds using a hand counter.

Assessments in Female Parents

Maternal Retrieval

Instinctual maternal responding was evaluated using the test of maternal retrieval on PND2 or PND3. Home cages were removed from the rack and placed on a hard surface. The dam was momentarily removed from the cage while 3 pups were taken from the nest and moved to the opposite end of the cage. The dam was immediately placed back in the cage. The latency for the dam to retrieve all three pups and return them to the nest was recorded in minutes using a standard stopwatch. If the dam had multiple nests, a single nest was created. Dams meeting this criterion and with failure to retrieve were retested with the nest moved at the opposite end of the home cage. Pups and dam were reunited if the pups were not retrieved in 5 minutes and a "timed out" designation (300 second performance) was recorded.

Assessments in Male and Female Parents and Adult Offspring

Motor Activity (MA)

Gross locomotor movements and exploratory behavior were evaluated in parents and offspring using a photobeam activity system (PAS) and software (SDI, San Diego, CA). Animals were individually placed in 16" (W) x 16" (D) x 15" (H) clear plastic open fields with horizontal and elevated photobeams that automatically recorded beam breaks using the PAS software. The activity meters had photocells aligned 1 inch apart to detect horizontal movement and vertical rears, as well as differentiate small (stereotypic) movements from large movements. Each apparatus was located in a room with white noise generated at 68 dB to mask ambient noise levels below 65 dB. Also, low level illumination was set at 30 lux. To begin the test, animals were placed in the center of the open field and left uninterrupted for the duration of the 30 minute test session. Between each test, all fecal boli were removed and the open fields were washed with a solution of 10% ethanol to remove any olfactory cues that may have been left behind. Measures recorded were: distance traveled (cm), active time/resting time (sec), average speed (cm/sec), number of beam breaks (stereotypical), number of rears, and percentage of time in center vs. perimeter.

Water-maze Navigation

The water-maze navigation was used to evaluate motor coordination, learning and spatial memory (Morris, 1984; Voorhees and Williams, 2006) on parents and a select number of their offspring. The maze was a 183cm diameter dark blue plastic tank (San Diego Instruments, San Diego, CA) 30cm high filled nearly to the top with water maintained at 22-25°C. A 10cm square clear escape platform large enough for the animal to stand on was attached to the floor of the tank, but submerged 1" below the surface of the water. Shiny visual cues of different shapes (i.e. triangle, square, circle, T) were located outside of the tank and designated for each quadrant of the tank. During training, the animal was placed in the water facing the wall, at 1 of

3 locations distal from the escape platform. Throughout training, animals were randomly placed into the different quadrants so that all quadrants equally served as start zones and no obvious pattern could be learned. The animal was allowed to swim until reaching the escape platform or until the 90 second time-out. The animal was removed from the tank, dried with a towel, given a 15 second rest period before the next trial. Animals were trained for 3 trials per day for five days until they could consistently swim to the platform in less than 20 seconds. On the final test day, 24 hours later, the platform was removed from the tank and a single 90 second probe trial was administered for each animal starting from the opposite corner quadrant. The total distance (cm) the animal swam and latency (sec) to find the platform were electronically recorded for 90 seconds using a SMART tracking system and water-maze software (San Diego Instruments). Additionally, on the probe trial day, percentage of time spent in the previous platform quadrant and the total number of crossings over the previous platform location were also recorded.

Results

Environmental Parameters

The whole body inhalation exposure system, which was developed specifically for this project, performed very well and proved the laboratory's capability to control test conditions within the parameters specified by the study protocol. The lone exception was the lower humidity in the control chamber as discussed above in Materials and Methods (Table 4). Based on the results of Phase 2 exposures, no alterations to the established mixed gas flow rates (Table 5) and the resulting mixed gas concentrations are deemed to be necessary to conduct Phase 3.

Deaths

No animal deaths occurred during the 28-day exposure period during Phase 2, and no animals were removed from their respective exposure test atmospheres beyond the 1-hour daily limit allowed for routine husbandry and performance of vaginal lavage, as described previously.

However, two deaths were recorded for the parental (P1) generation. A female from the control group was removed from the study during the mating phase after she developed a large tumor in her abdomen. She was euthanized in accordance with the study protocol, and necropsy revealed no other abnormalities. Given that the animal was not exposed to any of the mixed gas test atmospheres, this event cannot be attributable to exposure and is considered a background incident. No other females in the study developed tumors. Additionally, a female from the mid-dose group died either during parturition, or shortly thereafter. The cause of death was obstructive dystocia due to spontaneous metritis during parturition. Of eight pups that had been born, three were found dead, and the surviving five pups were lethargic. The surviving pups were euthanized in accordance with the study protocol. The Crl:CD(SD) rat has a high natural background incidence of vaginal infection (up to 6%) during pregnancy, which can result in the formation of an occlusive membrane of connective tissue called a vaginal septum. The presence of these septa often leads to severe mucus accumulation and dystocia (Lezmi, et al., 2011). Therefore, this incident is considered to be a normal background event of parturition.

Special Breeding Group

Evidence of successful exposure chamber mating was discovered in twelve females within four days of pairing, with litter size averaging 15 total pups after an average of 21.3 gestation days. The average weight per litter on PND0 was 95.1 grams. All of these values are comparable to normal historical values for this animal (Sharp and La Regina, 1998). Therefore, it is concluded that detrimental effects from exposure cages and/or inhalation chambers on the mating behavior and parturition for Phase 3 are not of concern.

Estrous Cycles

The difference between the proportions of time spent in each estrous cycle phase for each dose group in comparison to controls were compared using the Pearson Chi-square test (X^2, α=0.05).

There were no indications that exposures to the three test gas mixtures had any effect upon the estrous cycle, since female animals from the dose groups were observed to have estrous cycle phase proportions that are not statistically different from the control animals (Table 6).

Mating and Monitoring of Pregnancy and Offspring

The proportional differences between mating and delivery success rates of the dose groups in comparison to controls were determined using the Pearson Chi-square test (X^2, $\alpha = 0.05$). All differences in quantitative measures were determined using two-way analysis of variance (ANOVA). Dose groups with significant effects were identified using Dunnett's (post hoc) method for multiple comparisons. The critical values that are reported for the ANOVAs are the main effect degrees of freedom, error degrees of freedom, F-ratio, and the associated p-value. For X^2 tests the degrees of freedom, sample size and the X^2 statistic are reported. Pair-wise p-values are also reported, as well as p-values > 0.05 for certain critical study measures; however, only statistically significant values are presented in complete format.

P1 Generation

The gestation and parturition data across all dose groups and controls indicate that neither the maternal exposure levels, nor the paternal exposure levels are significant factors affecting reproductive success.

Mating success (indicated by the presence of a seminal plug or subsequent delivery of pups) was unaffected by exposure (maternal exposures: $p = 0.799$; maternal and paternal exposures; $p = 0.655$, X^2, Table 7). Success in delivery of litters was also unaffected by exposure (maternal exposures: $p = 0.283$; maternal and paternal exposures: $p = 0.719$, X^2, Table 7).

Other reproductive endpoints (Table 7) that were unaffected by exposure include: (1) the length of gestation (maternal exposures: p = 0.645; maternal and paternal exposures: p = 0.371, ANOVA); the stillbirth index (fraction of pups dead at birth; maternal exposures: p = 0.349; maternal and paternal exposures: p = 0.522, ANOVA); and, the sex ratio (male fraction of live pups; maternal exposures: p = 0.680; maternal and paternal exposures: p = 0.636, ANOVA). Litter size (live plus stillborn) was unaffected by maternal exposure level (p = 0.107), but was affected by paternal exposure level ([F (4, 203) = 3.16, p = 0.015], ANOVA). However, dose group comparisons found no significant differences between the numbers of pups sired by the control group in comparison to paternal dose groups (C < L, p = 0.335, Dunnett's Method).

The number of live pups per litter on PND0 was affected by both the maternal [F (3, 204) = 3.44, p = 0.018] and the paternal [F (4, 204) = 3.18, p = 0.015] exposure level (ANOVA); however, the dose group comparisons found no significant differences between the number of live pups born to the control dams in comparison to the dams from other dose groups (C < L, p = 0.087, Dunnett's Method) or between the number of live pups sired from the control group in comparison to paternal dose groups (C < L, p = 0.326, Dunnett's Method).

F1 Generation

The data across all dose groups and controls indicated that neither the maternal, nor paternal, exposure levels are significant factors affecting the basic development and growth in offspring.

The average pup body weight on PND0 was unaffected by maternal (females: p = 0.540; males: p = 0.627) or paternal (females: p = 0.918; males: p = 0.844) exposures (ANOVA, Table 8).

Other important developmental endpoints (Table 8) that were unaffected by exposure include: (1) the viability index (pups alive at PND4, as a fraction of live-born pups; maternal exposures:

p = 0.839; maternal and paternal exposures: p = 0.531, ANOVA); the lactation index (fraction of pups retained at the PND 4 cull surviving to PND21; maternal exposures: p = 0.165; maternal and paternal exposures: p = 0.386, ANOVA); and the average age for all pups in a litter to have their eyes and ears opened; maternal exposures: p = 0.838; maternal and paternal exposures: p = 0.308, ANOVA).

Female pup weight on PND21 was unaffected by exposure (maternal exposures: p = 0.072; maternal and paternal exposures: p = 0.852, ANOVA). Although male pup weight on PND21 was also unaffected by paternal (p = 0.866) exposure levels, male pup weight was affected by maternal [F (3, 201) = 2.99, p = 0.032] exposure levels (ANOVA). The PND21 body weight of male pups born to dams from the high- dose group was significantly greater than the weight of male pups born to dams from the control group (C < H, p=0.008, Dunnett's test). The body weight of male pups born to dams from the low- and mid-dose groups did not differ significantly from the controls (C < L, p = 0.149 and C < M, p = 0.170, respectively; Dunnett's test). The significance of weight differences between male pups from the high-dose group in comparison to controls at PND21 is not considered to be biologically significant, due to the fact that the significant body weight differences in all offspring had resolved by full growth at euthanasia (PND60+; p = 0.082, ANOVA, Table 9).

Body Weight/Body Weight Changes

Differences in P1 animal weight gains after 28 days of exposure were compared with one-way analysis of variance (ANOVA), with dose levels being the between group factor. All pair-wise comparisons for the ANOVAs were performed using Tukey–Kramer procedures. The critical values that are reported are the main effect degrees of freedom, error degrees of freedom, F-ratio, and the associated p-value. The specific pair-wise p-value is reported as well.

No statistically significant weight changes were observed in the exposed female rats (Table 10). Exposure had a statistically significant effect on weight loss in the mid-dose group for exposed males ([F (3, 108) = 8.90, p≤0.001, ANOVA]; C>M, p<0.001; L>M, p≤0.001); however, the low- and high-dose groups did not differ significantly from the controls (Table 11). The reason why this effect was observed in only the mid-dose group is not known; however, according to the criteria from the U.S. EPA Benchmark Dose Technical Guidance Document (U.S. EPA, 2000), the biological significance of weight changes ≤ 10% between treatment and control groups is not considered noteworthy. Tables 10 and 11 show no weight changes greater than 7.6%.

Tissue Weights

Organ weight differences were determined by an analysis of covariance (ANCOVA). Effects examined were the dose group and the status of paternal exposure (exposed or unexposed). For all tests performed, weight was considered the covariate. Data were checked with Levene's test for equality of variances. Homogeneity of slopes (HOS) was determined by looking for non-statistically significant interaction terms. If the slopes were not significantly different from each other for both factors, then the ANCOVA was run. If the slopes were significantly different, then the McSweeney-Porter method was used to convert the response and covariate into ranks before the ANCOVA was run. The critical values that are reported are the main effect degrees of freedom, error degrees of freedom, F-ratio, and the associated p-value.

P1 Generation

There were no statistically significant differences between the mean weights of organs (kidney, spleen, liver and ovary or testes) taken from exposed rats at necropsy in comparison to controls (Tables 12 & 13).

F1 Generation (PND 24-35)

There were no significant differences observed between the mean organ weights (brain, heart, kidney, spleen, liver, and ovary or testes) of tissues taken from juvenile offspring at necropsy in comparison to controls that were related to paternal exposure status. There was a significant decrease in mean ovary weight in juvenile female offspring of the low-, mid-, and high-dose group dams ([F (3, 56) = 3.71, p = 0.017], Tables 14 & 15). However, there were no statistically significant organ weight differences between male offspring of exposed dams in comparison to controls (Tables 16 & 17).

F1 Generation (PND 60-74)

There were no significant differences observed between the mean organ weights (brain, heart, kidney, spleen, liver, and ovary or testes) of tissues taken from adult offspring at necropsy in comparison to controls that were related to paternal exposure status. There was a significant decrease in the mean heart weight in adult female offspring of low-, mid-, and high-dose group dams ([F (3, 55) = 7.61, p ≤ 0.001], Tables 18 & 19). However, there were no significant organ weight differences between male offspring of exposed dams in comparison to controls (Tables 20 & 21).

Hematology

Differences in hematology values between the dose groups were determined by a one-way ANOVA when data were found to be normal or by a non-parametric one-way Kruskal-Wallis (KW) test when data normality failed the Shapiro-Wilk test ($p < 0.05$). Pair-wise comparisons for the ANOVAs were performed using Tukey–Kramer procedures, or by using Conover-Inman procedures for KW tests. Critical values reported for the ANOVAs are the main effect degrees of freedom, error degrees of freedom, F-ratio, and the associated p-value. For the KW tests the degrees of freedom and the H statistic are reported. All pair-wise p-values are also reported.

P1 Generation

The only significant difference that was observed in the directly exposed animals in comparison to controls was a small increase in monocyctes as a percentage of total WBCs in the high-dose group males ([F (3, 61) = 4.50, p = 0.027, ANOVA], C < H, p = 0.024, Tables 22 & 23).

F1 Generation (PND 24-35)

There were no statistically significant hematological differences observed in juvenile offspring related to paternal exposure status. However, there were statistically significant hematopoietic differences observed in juvenile offspring between the maternal dose groups and the controls. Statistically significant increases in the RBC count in comparison to controls was observed in female pups of high-dose group dams ([H (3, 10.60) = 0.014, KW], C < H, p = 0.005, Table 24). There was also a significant increase in the hemoglobin levels observed in female pups of high-dose group dams ([H (3, 9.20) = 0.027, KW], C < H, p = 0.013, Table 24) and male pups of mid- and high-dose group dams ([F (1, 20) = 28.59, p = 0.004, ANOVA], C < M, p = 0.040; C < H, p = 0.012, Table 25). Proportional increases were observed for the MCH and MCHC measures of hemoglobin. In addition, significant WBC increases in comparison to controls were observed in female pups of mid- and high-dose group dams ([F (3, 61) = 3.69, p = 0.016, ANOVA]; C < M, p = 0.027; C < H, p = 0.031, Table 24). Elevated WBC counts were also observed in male pups. The most prominent difference between maternal dose groups in comparison to controls was a general increase in platelet concentrations observed in the female ([H (3, 15.94) = 0.001, KW], C < L, p = 0.030; C < M/H, p ≤ 0.001, Table 24) and the male ([H (3, 16.5) = 0.001, KW], C < L, p = 0.024; C < M/H, p ≤ 0.001, Table 25) offspring of the low-, mid-, and high-dose group dams.

F1 Generation (PND 60-74)

Similar hematopoietic effects as observed in the juvenile offspring were also found in the adult offspring. Significant increases in red blood cell (RBC) count in comparison to controls was

observed in the adult female offspring of low-, mid-, and high-dose group dams ([H (3, 20.71) = 0.001, KW], C < L/M/H, p ≤ 0.001, Table 26) and the adult male offspring of high-dose group dams ([H (3, 8.86) = 0.031, KW], C < H, p = 0.003, Table 27). There was a significant increase in hemoglobin levels observed in adult female offspring of low-, mid-, and high-dose group dams ([H (3, 29.22) = 0.001, KW], C < L/M/H, p ≤ 0.001, Table 26) and adult male offspring of mid- and high-dose group dams ([H (3, 21.45) = 0.001, KW], C < M/H, p ≤ 0.001, Table 27). Proportional increases were also observed for the MCH and MCHC measures of hemoglobin. Additionally, a significant increase in percent hematocrit was observed in adult female offspring of low-, mid-, and high-dose group dams ([H (3, 22.91) = 0.001, KW], C < L/M/H, p ≤ 0.001, Table 26) and adult male offspring of high-dose group dams ([H (3, 7.80) = 0.05, KW], C < H, p = 0.006, Table 27).

All dose groups had observed white blood cell (WBC) increases for most types of leukocytes. Significant WBC increases in comparison to controls were observed in adult female offspring of low-, mid-, and high-dose group dams ([F (3, 54) = 14.34, p ≤ 0.001, ANOVA], C < L/M/H, p ≤ 0.001, Table 26) and adult male offspring of high-dose group dams ([H (3, 9.31) = 0.025, KW], C < H, p = 0.005, Table 27). Also, a decrease in lymphocytes as a percentage of total WBCs was observed for all dose groups in comparison to controls and was statistically significant in adult female offspring of low-, mid-, and high-dose group dams ([F (3, 52) = 12.71, p ≤ 0.001, ANOVA], C < L/M/H, p ≤ 0.001, Table 26); and adult male offspring of mid- and high-dose group dams (F (3, 58) = 5.51, p = 0.002, ANOVA], C < M, p = 0.003; C < H, p = 0.016, Table 27). Significant increases in platelets were observed in adult female offspring of low-, mid-, and high-dose group dams ([H (3, 20.43) = 0.001, KW], C < L/M/H, p ≤ 0.001, Table 26) and adult male offspring of mid- and high-dose group dams ([H (3, 11.94) = 0.008, KW], C < M/H, p = 0.002, Table 27).

Serum Chemistry

Differences in clinical chemistry values between dose groups were determined by a one-way ANOVA when data were found to be normal or by a non-parametric one-way Kruskal-Wallis (KW) test when data normality failed the Shapiro-Wilk test ($p < 0.05$). Pair-wise comparisons for the ANOVAs were performed using Tukey–Kramer procedures, or by using Conover-Inman procedures for the KW test. The critical values that are reported for the ANOVAs are the main effect degrees of freedom, error degrees of freedom, F-ratio, and the associated p-value. For KW tests the degrees of freedom and the H statistic are reported. Specific pair-wise p-values are also reported.

P1 Generation

The only significant difference that was observed in the directly exposed animals in comparison to controls was a decrease in the blood urea nitrogen in the high-dose group males ([$F (3, 35) = 4.25$, $p = 0.012$, ANOVA], C > H, $p = 0.018$, Tables 28 & 29).

F1 Generation (PND 24-35)

There were no statistically significant differences in serum chemistry values in juvenile offspring related to paternal exposure status. However, there were some minor changes identified based on maternal exposure status, including: (1) decreased blood glucose concentrations in juvenile offspring of exposed dams, which effect was statistically significant in female ([$F (3, 52) = 13.41$, $p \leq 0.001$, ANOVA], Table 30) and male ([$F (3, 52) = 14.79$, $p \leq 0.001$, ANOVA], Table 31) pups of high-dose group dams; and, (2) significantly decreased serum aspartate transaminase (AST) levels in juvenile female offspring of low-, mid- and high-dose group dams ([$H (3, 12.37) = 0.006$, KW], C > L, $p = 0.002$; C > M, $p \leq 0.001$; C > H, $p = 0.023$, Table 30) and male offspring of low- and mid-dose group dams ([$H (3, 11.89) = 0.008$, KW], C > L, $p = 0.010$; C > M, $p \leq 0.002$, Table 31).

F1 Generation (PND 60-74)

There were no statistically significant differences in serum chemistry values in adult offspring related to paternal exposure status. However, there were some minor differences identified that were based on sex and maternal exposure status, including: (1) decreased potassium ion concentrations in adult female offspring of low-, mid- and high-dose group dams compared to controls ([H (3, 15.58) = 0.001, KW], C < L, p = 0.037; C < M/H, p ≤ 0.001, Table 32); and, (4) a decrease in blood urea nitrogen (BUN) in adult male offspring of low-, mid-, and high-dose group dams compared to controls ([F (3, 35) = 4.25, p = 0.001, ANOVA], C > L, p = 0.014; C > M/H, p ≤ 0.001, Table 33).

Histopathology

Differences between the proportion of rats exhibiting specific pathologies for each dose group were compared using the Pearson Chi-square test (X^2, α=0.05) and all post-hoc comparisons were performed using Fisher's exact test for pair-wise comparisons. All proportions were based on incidence data only, and not the severity of a histological finding. The critical values that are reported for X^2 tests are the degrees of freedom, sample size, and the X^2 statistic. Pair-wise p-values are also reported, as well as p-values > 0.05 for certain critical study measures. Only statistically significant values are presented in complete (APA) format. Tissues from control and dose groups were prepared for microscopic examination following necropsy. A detailed report of histopathological findings is included as Appendix A.

P1 Generation

There were no pathological observations identified in the adrenal glands, epididymis, ovaries, oviducts, spleen or testes. A few idiopathic, statistically non-significant, findings were observed in the brain, heart, liver, pancreas and pituitary gland, which data indicate were incidental to the exposure due to a lack of dose response and the fact that all other evaluated sections of these

tissues were normal. Exposure had a significant effect on the incidence of abnormal kidney findings in exposed females ([X^2 (3, 9) = 0.030], Table 34). A pair-wise comparison determined that the mid-dose females had a significantly lower incidence of abnormal kidney findings than the controls (p = 0.032, Fisher's exact test), while the low- and high-dose groups did not differ significantly from controls (p = 0.458, Fisher's exact test). A lesion specific analysis indicated a dose-related difference in kidney fibrosis [X^2 (3, 9.44) = 0.024]; however, pair-wise comparison indicated that no significant dose related differences from controls were identifiable (p = 0.119, Fisher's exact test). No significant effects on the incidence of any lesions were identified in the exposed male rats (Table 35).

F1 Generation (evaluated at PND 21-23)

There were no significant differences in the incidence of lesions related to paternal exposure status. There were no pathological findings identified in the brain, heart, liver, mammary glands, ovaries, oviducts, pancreas, spleen or testes. A few idiopathic, non-significant, pathological findings were observed in the adrenal glands, epididymis, pituitary gland, uterus and uterine horns; but the data indicate that these findings were incidental to exposure due to a lack of dose response and the fact that other evaluated sections of these tissues were normal. Parental exposure had a significant effect on the total incidence of abnormal kidney findings in female pups born to exposed parents [X^2 (3, 8.64) = 0.035], with fewer findings in the offspring of the exposed animals in comparison to controls (Tables 36 & 37). Pair-wise comparisons showed that no significant differences existed between offspring based on dose (p = 1.000, Fisher's exact test). In addition, the level of parental exposure did not show a significant effect on the incidence of any specific kidney lesion (e.g., kidney cysts, [X^2 (3, 6.67) = 0.083]). There was no significant effect on the incidence of any organ abnormalities in exposed males in comparison to controls (Tables 38 & 39).

F1 Generation (evaluated at PND 60+)

There were no pathologies identified in the adrenal glands, brain, mammary glands, ovaries, oviducts or pituitary gland. Idiopathic, statistically non-significant, pathological findings were observed in the epididymis, heart, liver, pancreas, spleen, testes, uterus and uterine horns; however, the data indicate that the findings were incidental to exposure due to a lack of dose response and the fact that other evaluated sections of these tissues were normal. There was no significant effect on the incidence of any organ abnormalities in the female adult offspring (Tables 40 & 41) in comparison to controls. There was a significantly higher incidence of kidney fibrosis in the male offspring of exposed dams and sires [X^2 (3, 9.93) = 0.029, Table 42]. However, a pair-wise comparison showed no significant differences between the male offspring based on dose in comparison to the offspring of the controls (p = 0.077, Fisher's exact test). Paternal exposure had a significant effect on the total incidence of abnormal kidney findings in male offspring of exposed dams and unexposed sires ([X^2 (3, 12.19) = 0.007], Table 43). Pair-wise comparison showed that the offspring of dams from the high-dose group had a significantly higher incidence of abnormal kidney findings in comparison to offspring of controls (p = 0.041, Fisher's exact test). However, a lesion-specific assessment did not indicate any significant differences related to maternal dose (chronic infiltrates, [X^2 (3, 4.65) = 0.200]), suggesting that the effect was a statistical artifact of combining independent variables with unrelated pathology.

Neurobehavioral Assessments

Differences in neurobehavioral measures between dose groups were determined by a two-way ANOVA when data were found to be normal, or by a non-parametric one-way Kruskal-Wallis (KW) test when data normality failed the Shapiro-Wilk test ($p<0.05$). Pair-wise comparisons for the ANOVAs were performed using Tukey–Kramer procedures, or by using Conover-Inman procedures for KW tests. Critical values reported for the ANOVAs are the main effect degrees

of freedom, error degrees of freedom, F-ratio, and the associated p-value. For the KW tests the degrees of freedom and the H statistic are reported. All pair-wise p-values are also reported.

All exposed parents and a subset of their offspring were evaluated for neurobehavioral effects using a small battery of neurobehavioral tests selected from the neurotoxicity assessment battery (NTAB) developed in our laboratory (Arfsten et al., 2009). No significant dose related effects were found for the exposed parents, nor for adult offspring that received the same adult tests as their parents. Minimal dose-related effects were observed in juvenile offspring under both paring conditions (female + male parent exposed or female parent exposed + male parent unexposed) for early developmental tests.

P1 Generation

Motor Activity

No significant differences between dose groups and controls were detected in parents for any of the motor activity measurements. Total distance, activity time, average speed, total rears and percentage of time in center vs. perimeter of the test field yielded similar results between groups (Table 44).

Water-maze Learning and Memory

No significant dose-related effects were observed on any day during the 5-day learning phase of water-maze navigation between dose groups or in comparison to controls, nor during the 24 hour probe trial to test the memory function of exposed rats (Table 44).

Maternal Retrieval

Latency to retrieve offspring for high-dose group dams was significantly longer than for dams from the other dose groups or controls ([F (3, 56) = 3.44], p = 0.023, ANOVA), but only for the

offspring sired by exposed males (both parents exposed) and not for offspring having maternal exposure only (Table 44).

F1 Generation (Pup Tests)

Righting Reflex

A natural sex effect, independent of exposure, is a demonstrated tendency for male rats to right themselves faster than females. The only statistically significant effect related to parental exposure, and independent of sex differences, was an increased latency of offspring from the mid-dose group to right themselves in comparison to offspring from the high-dose group ([F (3, 245) = 2.95, p = 0.009, ANOVA], Table 45). This effect was only significant in offspring of exposed dams and unexposed sires, and does not appear to be a dose response.

Separation Distress

The only significant effect related to parental exposure was a decrease in distress vocalizations (calls for help from the pup to the dam) from the female offspring of parents from the high-dose group in comparison to controls ([H (3, 11.34) = 0.01, KW], Table 45).

F1 Generation (Adult Tests)

Motor Activity

No significant differences between dose groups or controls were detected in parents for any of the motor activity measurements. Total distance, activity time, average speed, total rears and percentage of time in center vs. perimeter of the test field yielded similar results between groups (Table 46).

Water-maze Learning and Memory

No dose-related effects were observed on any day of the 5-day learning phase of water-maze navigation except on day 4, when the latency of the offspring from parents in the low-dose group were significantly shorter than the offspring from parents in the mid- and high-dose groups, but not controls [F (3, 28) = 5.00, p = 0.007, ANOVA]. Given that this change in performance was not repeated during the learning test cycle and no significant dose-related effects were observed between the groups during the 24 hour probe trial to test the memory function, this effect is not biologically significant (Table 46).

Discussion

The purpose of this phase of the study was to evaluate potentially adverse submarine atmosphere exposure effects on neurological and reproductive performance. All evaluated measures of reproduction and development were found to be within normal historical ranges and statistically non-significant with respect to controls after 28 days of exposure. Neurological performance testing revealed no associated effects between exposure status (parental exposure) and any change in early motor development, activity levels and exploratory behaviors, or higher-level cognitive functions (learning and memory) in either parents or offspring. Two subtle differences were discovered during the parent and offspring tests on emotionality, including an increased latency in the maternal retrieval of pups by dams from the high-dose group in comparison to controls, and a decrease in ultrasonic distress vocalizations (calls for help from the pup to the dam) by female pups born to dams from the same group, which may account for the decreased awareness of the dam to locate her pups. Because the only significant effects were related to emotional developmental, Phase 3 analysis will include juvenile play (Ikemoto and Panksepp, 1992) as an additional measure to assess emotional response activities in animal development.

Additional measures of development and general health were largely unremarkable. The gross necropsy, clinical chemistry, tissue weights, and histopathology of the parent population (dose groups and controls) and their offspring at key stages of development revealed no significant evidence of adverse toxicity effects related to the mixed gas exposures. Statistically significant differences in weight and weight change between dose groups in comparison to controls, and between their offspring, were isolated, temporary, and well below the U.S. EPA's standard for biological significance. The reduction in heart weight for adult offspring of exposed parents was in direct contrast to the increased heart weights found in historical carbon monoxide studies of offspring exposed *in utero*. This effect, as well as the observed decrease in ovary weight, will be closely examined in Phase 3 to determine whether these effects persist and are adverse.

Observations of early stage chronic progressive nephropathy and other degenerative kidney processes commonly found in this strain of rat (Turnbull et al., 1985) continue to be noted in histopathological examinations of tissues across most groups. The cause of this disease is multi-factorial, but protein overload is the most common cause, with the proximal convoluted tubule being the most common site for this lesion. The clinical severity of these lesions was minimal, with no evidence of ischemia or hypoxia present in examined sections. Moreover, the frequency of the lesions was associated with increasing age. These findings are considered to be incidental to the mixed gas exposures, since all statistically significant differences found in the mean percentage incidence of renal tubular degeneration were higher in the controls than in the exposure groups. Additionally, there were observations of small clusters of infiltrates in the hepatic sinusoids of the liver across nearly all groups, including controls. As with nephropathy, the background incidence of this minimal finding is naturally high for this particular strain of rat, and there was no statistically significant increase in the proportion of [parentally] exposed rats affected compared to controls. Additionally, clinical chemistry analysis of the serum in offspring indicated that the enzyme used as the primary biomarker for liver damage (AST) was found at

much higher concentrations in offspring of control animals than in offspring of exposed animals. Therefore, at this time, these kidney and liver disease processes are currently considered to be spontaneous and incidental to the mixed gas exposures.

The lack of significant blood findings in directly exposed animals indicates that blood and serum effects from the mixed gas exposures appear to be reversible with a moderate recovery period. Several mild hematological and blood serum effects were observed during Phase 1 of this study (Hardt et al., 2011). However, since blood was drawn from the animals at necropsy, which for Phase 2 was three to five weeks post-exposure for sires and five to eight weeks post-exposure for dams, it is reasonable to assume that short-lived exposure effects may have been resolved before blood analysis.

Serum chemistry results for exposed rats were unremarkable in comparison to controls, with the exception of a decrease in blood urea nitrogen in the high dose group males, which also was a significant finding in male offspring that were sired by this same dose group. Significant findings identified in offspring include a decrease in glucose concentrations in the high-dose group pups in comparison to controls, which was in contrast to the effect in exposed rats from Phase 1; and, an increase in potassium ion concentrations across adult female dose groups in comparison to controls, which was a common finding for exposed male and female rats from Phase 1. Blood chemistry results are not indicative of any adverse effects in the offspring of exposed parents. Statistically significant increases in hematopoietic activity (mild polycythemia, leukocytosis and thrombocytosis) were observed in offspring of exposed parents compared to offspring of control animals, with the most pronounced effects observed in the adult offspring (PND60+, Tables 25 & 26). Polycythemia (increased red blood cells, with expected increases in hemoglobin and hematocrit) is an established effect of hypoxia. However, this effect occurred in offspring that were not directly exposed to the mixed gas test atmospheres. Leukocytosis (increased white

blood cells) and thrombocytosis (increased platelets) are typically signs of relatively benign conditions of infection or inflammatory processes. The normal reaction of the bone marrow to inflammation leads predominantly to skewed increases ("left shift") favoring polymorphonuclear leukocytes (less mature WBCs involved in innate immunity). This fact is consistent with findings of statistically significant decreases in lymphocytes as a percentage of total WBCs for the adult offspring of exposed parents compared to offspring of controls. Physical and emotional stress can also elevate white blood cell counts, as well as possible bone marrow disorders. However, it must be emphasized that although these hematological effects were statistically significant, all mean values for these parameters fell within, or very close to, the normal ranges established for these animals. Additionally, histopathological examination of livers and spleens in the rats failed to confirm clinical significance to the hematological findings, as did a lack of enzymatic activity in the blood serum chemistries. The etiology of these hematopoietic effects is currently unknown and will be examined further in Phase 3 of the study by additional analysis using immunoassays for applicable hormones (i.e. erythropoietin), cytokines (i.e., interleukin (IL)-1; IL-3; substance P) and other pertinent biomarkers, such as erythroid burst-forming units (BFC-E) and granulocyte-macrophage colony forming units (GM-CFU).

While it is known that hypoxia can affect the proliferation and control of GM-CFU and BFC-E in bone marrow stroma by induction of the preprotachykinin-I (PPT-I) gene (Quinlan et al, 1998), it may be possible for this PPT-I induction to occur in the unexposed offspring of exposed dams through an unknown maternal mediator(s), possibly substance P. It is anticipated that additional tests to be performed during Phase 3 may help clarify whether the present results indicate a low level condition of chronic inflammation or another cause (i.e., epigenetic effects, a bone marrow disorder, etc.). It is also possible that these results are simply an analytical or statistical artifact, since several important hematological endpoints for the control group have lower than expected values that may purport errors in phlebotomy techniques in collecting blood (Lewis et al, 1985).

One unique aspect of this study was the addition of extra groups of F1 offspring to determine whether paternal exposure, in addition to maternal exposure, played a role in any particular effect. Paternal exposure was implicated in only one instance, when the offspring of parents where only the dam was exposed exhibited an effect that was statistically significant from the results presented in offspring of parents that were both exposed. The effect was an increased latency by the offspring of mid-dose group dams to reflexively right themselves in comparison to offspring from high-dose group dams. Since there is no apparent dose-response, this effect is currently not considered to be an outcome of exposure, but will be further examined in Phase 3.

In conclusion, 28-day exposures to elevated concentrations of the mixed submarine atmosphere gases described did not affect the ability of rats to reproduce and did not result in any significant developmental deficits in their offspring.

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Table 1: Parameters Measured for P1 and F1 Generations

Generation	Parameter
P1	Weight Gain While Under Exposure
	Estrus cycle
	Mating Success
	Delivery Success
	Gestation Length
	F1 PND0 Litter Size (Total Pups and Live Pups)
	F1 PND0 Litter Weight (Male and Female)
	F1 PND0 Sex Ratio
	Maternal Care of Young
	Neurobehavioral Assessments
	Hematology
	Serum Chemistries
	Post-necropsy Tissue Weights
	Histopathology of Target Tissues
F1	Gross Malformations PND1-4
	PND 1-21 Weight Gain
	PND1-21 Survival Rates
	Neurobehavioral Assessments PND3-8
	PND4 Survival
	Days to eyes and ears open
	Hematology
	Serum Chemistries
	Neurobehavioral Assessments PND51+
	Post-necropsy Tissue Weights
	Histopathology of Target Tissues

Table 2: P1 Mating and Exposure Groups

Mating Group	Sex	Exposure Conditions
Group 1 (C/C)	Female	Control Atmosphere (28 days in exposure chamber)
	Male	Control Atmosphere (28 days in exposure chamber)
Group 1a (C/U)	Female	Control Atmosphere (28 days in exposure chamber)
	Male	Naive (unexposed to test atmospheres or exposure chambers)
Group 2 (L/L)	Female	Low Dose Atmosphere (28 days in exposure chamber)
	Male	Low Dose Atmosphere (28 days in exposure chamber)
Group 2a (L/U)	Female	Low Dose Atmosphere (28 days in exposure chamber)
	Male	Naive (unexposed to test atmospheres or exposure chambers)
Group 3 (M/M)	Female	Mid Dose Atmosphere (28 days in exposure chamber)
	Male	Mid Dose Atmosphere (28 days in exposure chamber)
Group 3a (M/U)	Female	Mid Dose Atmosphere (28 days in exposure chamber)
	Male	Naive (unexposed to test atmospheres or exposure chambers)
Group 4 (M/U)	Female	High Dose Atmosphere (28 days in exposure chamber)
	Male	High Dose Atmosphere (28 days in exposure chamber)
Group 4a (H/H)	Female	High Dose Atmosphere (28 days in exposure chamber)
	Male	Naive (unexposed to test atmospheres or exposure chambers)

Table 3: Neurobehavioral Testing Schedule.

Test	Number of Animals	Day
Maternal Retrieval (P1 – Dams)	32 Dams with EE [a] Litters 32 Dams with EU [b] Litters	PND 2 or 3
1st Litter Cull – PND4 (Retained Pups: 4 Males + 4 Females per Litter)		
Righting Reflex (F1 – Offspring)	256 Pups from EE [a] Litters (32 litters) 256 Pups from EU [b] Litters (32 litters)	PND 4 or 5
Separation Distress (F1 – Offspring)	256 Pups from EE [a] Litters (32 litters) 256 Pups from EU [b] Litters (32 litters)	PND 7 or 8
2nd Litter Cull (Weaning) – PND19-23 (Retained Pups: 1 Male + 1 Female per Litter)		
Motor Activity and Watermaze (P1 – Parents)	32 Exposed Sires 32 Exposed Dams	PND 19-23
Motor Activity and Watermaze (F1 – Offspring)	64 Adults from EE [a] Litters 64 Adults from EU [b] Litters	30-60 Day Post Exposure
Motor Activity and Watermaze (F1 – Offspring)	64 Adults from EE [a] Litters 64 Adults from EU [b] Litters	60-90 Day Post Exposure

[a] Exposed Dam + Exposed Sire

[b] Exposed Dam + Unexposed Sire

Table 4: Inhalation exposure summary data: environmental parameters

			Group 1 Control	**Group 2** Low Dose	**Group 3** Mid Dose	**Group 4** High Dose
Temperature	(Deg C)	Mean	21.8	24.9	24.5	24.6
		Std Dev	1.2	0.5	0.5	0.5
		Min	21.2	24.6	23.9	24.2
		Max	24.3	26.1	25.2	25.4
		Count	28	28	28	28
Humidity	(%)	Mean	22	57	44	46
		Std Dev	4	5	5	5
		Min	13	47	35	38
		Max	29	65	54	60
		Count	28	28	28	28
Supply Air Flow Rate	(L/min)	Mean	392	185	172	164
		Std Dev	11	4	2	6
		Min	348	181	169	159
		Max	406	198	175	180
		Count	28	28	28	28
Carbon Monoxide Concentration	(ppm)	Mean	0 .38	5.0	13.7	85.6
		Std Dev	0 .01	0.6	1.4	7.7
		Min	0.36	2.8	7.2	48.6
		Max	0 .41	5.9	14.6	91.3
		Count	28	28	28	28
Carbon Dioxide Concentration	(%)	Mean	0.10	0.44	1.15	2.35
		Std Dev	0.03	0.01	0.14	0.36
		Min	0.08	0.41	0.52	0.82
		Max	0.17	0.46	1.24	2.52
		Count	28	28	28	28
Oxygen Concentration	(%)	Mean	20.4	16.9	15.9	14.9
		Std Dev	0.3	0.3	0.4	0.2
		Min	19.8	16.6	15.5	14.5
		Max	20.9	18.2	17.4	15.3
		Count	28	28	28	26
Static Pressure	(in H2O)	Mean	-0.01	-0.09	-0.10	-0.09
		Std Dev	0.00	0.06	0.03	0.06
		Min	-0.02	-0.31	-0.16	-0.34
		Max	-0.01	-0.01	-0.05	-0.01
		Count	28	28	28	28

Table 5: Inhalation exposure summary data: test chemical flow rates

			Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Carbon Monoxide Flow Rate	(mL/min)	Mean	1.1	2.7	14.7
		S Dev	0.2	0.3	1.4
		Min	0.8	1.6	7.8
		Max	1.4	3.1	16.1
		Count	27 [a]	28	28
Carbon Dioxide Flow Rate	(L/min)	Mean	0.44	1.93	4.72
		S Dev	0.02	0.32	0.82
		Min	0.41	0.56	1.16
		Max	0.49	2.24	5.24
		Count	27 [a]	28	28
Nitrogen Flow Rate	(L/min)	Mean	34.0	45.2	47.9
		S Dev	3.2	4.0	4.7
		Min	18.6	24.8	26.7
		Max	35.0	47.1	49.9
		Count	27 [a]	28	28

[a] National Instruments data for Day 28 was inadvertently deleted

Table 6: Summary of estrus cycle monitoring data*

Estrus Cycle Phase	Group 1 Control $n = 8_{[a]}; 40_{[b]}$	Group 2 Low Dose $n = 7_{[a]}; 41_{[b]}$	Group 3 Mid Dose $n = 8_{[a]}; 55_{[b]}$	Group 4 High Dose $n = 7_{[a]}; 54_{[b]}$
Proestrus	2.5 (6.25%)	1.5 (3.67%)	5 (9.09%)	3.5 (6.48%)
Estrus	15.5 (38.75%)	17.5 (42.7%)	23.5 (42.7%)	23.5 (43.5%)
Metestrus and Diestrus	22 (55.0%)	22 (53.7%)	26.5 (48.2%)	27 (50.0%)

[a] value indicates the number of rats per group

[b] value indicates the total number of observations per group

* Data were collected for 4 consecutive days, interrupted by a 3-day break, and then collected for an additional 1 to 4 days. Only dams that successfully delivered pups were included in this data summary.

Table 7: Gestation and parturition data (±SD) for all dose groups; n=(x).

Endpoint (Historical*)	**Group 1 (C/C)**	**Group 1a (C/U)**	**Group 2 (L/L)**	**Group 2a (L/U)**	**Group 3 (M/M)**	**Group 3a (M/U)**	**Group 4 (H/H)**	**Group 4a (H/U)**
Mating Rate (93% ± 10%)	100 (27)	100 (27)	100 (28)	96.4 (26)	100 (27)	96.4 (26)	96.4 (26)	100 (27)
Delivery Rate (97% ± 10%)	96.4 (26)	100 (27)	100 (28)	92.8 (25)	92.8 (25)	89.3 (25)	96.4 (26)	96.4 (26)
Gestation Period (22.3 days)	21.7 ± 0.5 (26)	21.9 ± 0.5 (21)	21.8 ± 0.5 (26)	21.8 ± 0.4 (24)	22.0 ± 0.4 (22)	21.8 ± 0.5 (21)	21.9 ± 0.5 (20)	21.6 ± 1.1 (17)
Litter Size (14.7 Pups)	15.3 ± 2.4 (27)	14.6 ± 2.2 (27)	15.6 ± 2.2 (28)	**13.7Ŧ ± 2.3** (26)	14.1 ± 1.6 (26)	14.0 ± 2.1 (24)	15.1 ± 1.8 (26)	15.0 ± 2.2 (27)
Live Pups per Litter (14.5 Pups)	15.2 ± 2.4 (27)	14.3 ± 2.4 (26)	15.1 ± 2.6 (28)	**13.0Ŧ ± 3.6** (26)	13.7 ± 1.8 (26)	13.6 ± 2.2 (24)	14.9 ± 1.8 (26)	15.0 ± 2.2 (27)
Stillborns per Litter (0.29 Pups)	0.1 ± 0.4 (27)	0.3 ± 0.7 (26)	0.5 ± 0.8 (28)	0.7 ± 2.3 (26)	0.4 ± 0.9 (26)	0.4 ± 0.6 (24)	0.2 ± 0.4 (26)	0.1 ± 0.2 (27)
Sex Ratio (50% males)	52.3 ± 12.6 (27)	52.5 ± 11.3 (27)	50.3 ± 12.0 (28)	48.9 ± 14.4 (25)	51.8 ± 14.3 (26)	48.9 ± 14.4 (24)	54.9 ± 9.7 (26)	50.6 ± 14.5 (27)

* Sharp, P., La Regina, M. The Laboratory Rat, CRC Press LLC, 1998

See Table 2 for description of groups

Ŧ Pearson Chi-square test indicated statistically significant differences (decreases in litter size and live births) between the low dose group and controls; however, Dunnett's Method of pair-wise comparisons showed that there were no statistically significant differences between the numbers of live pups born to control dams in comparison to the dams from other dose groups.

Table 8: Developmental Data (±SD) for F1 offspring; n=(x).

Endpoint (Historical*)	**Group 1 (C/C)**	**Group 1a (C/U)**	**Group 2 (L/L)**	**Group 2a (L/U)**	**Group 3 (M/M)**	**Group 3a (M/U)**	**Group 4 (H/H)**	**Group 4a (H/U)**
Viability Index (% living at PND4)	93 ± 12 (27)	94 ± 10 (26)	92 ± 9 (28)	96 ± 6 (24)	94 ± 11 (26)	96 ± 5 (25)	96 ± 10 (26)	97 ± 5 (27)
Lactation Index (% living at PND21)	99 ± 4 (27)	92 ± 23 (26)	97 ± 6 (27)	98 ± 7 (25)	95 ± 20 (26)	96 ± 12 (25)	99 ± 4 (26)	99 ± 3 (27)
Time to open eyes and ears (10 – 14 days)	14.7 ± 0.8 (27)	14.6 ± 0.8 (27)	14.3 ± 0.6 (28)	14.6 ± 0.6 (25)	14.4 ± 0.6 (25)	14.7 ± 0.9 (24)	14.7 ± 0.5 (25)	14.5 ± 0.8 (28)
Male Weight at PND0 (6.70 ± 0.37 grams)	6.7 ± 0.5 (27)	6.7 ± 0.5 (27)	6.8 ± 0.6 (28)	6.9 ± 0.7 (25)	6.7 ± 0.5 (26)	6.8 ± 0.7 (24)	6.6 ± 0.7 (26)	6.7 ± 0.6 (27)
Female Weight at PND0 (6.31 ± 0.36 grams)	6.3 ± 0.5 (27)	6.3 ± 0.6 (27)	6.4 ± 0.5 (28)	6.5 ± 0.6 (25)	6.3 ± 0.5 (26)	6.4 ± 0.5 (24)	6.3 ± 0.6 (26)	6.3 ± 0.6 (27)
Male Weight at PND21 (56.8 ± 5.9 grams)	56.9 ± 7.0 (27)	56.0 ± 7.5 (27)	59.9 ± 5.6 (28)	59.5 ± 5.5 (25)	61.2 ± 8.3 (25)	59.4 ± 7.2 (24)	60.7 ± 6.0 (26)	**61.5Ŧ± 7.3** (27)
Female Weight at PND21 (54.5 ± 5.6 grams)	54.6 ± 5.4 (27)	53.6 ± 6.9 (27)	57.6 ± 4.6 (28)	57.5 ± 6.5 (25)	57.9 ± 7.4 (25)	56.5 ± 7.1 (24)	58.0 ± 6.2 (26)	57.6 ± 6.3 (27)

* Sharp, P., La Regina, M. The Laboratory Rat, CRC Press LLC, 1998

See Table 2 for description of groups

Ŧ Two-way ANOVA indicated statistically significant differences (greater weight at PND21) between the high dose group and controls. Dunnett's Method of pair-wise comparisons showed that there was a statistically significant difference between the PND21 body weights of male pups born to dams from the high dose group in comparison to male pups born to dams from the control group.

Table 9: Terminal Body Weight Data at Euthanasia (±SD) for F1 offspring in grams; n=8.

Endpoint	Group 1 (C/C)	Group 1a (C/U)	Group 2 (L/L)	Group 2a (L/U)	Group 3 (M/M)	Group 3a (M/U)	Group 4 (H/H)	Group 4a (H/U)
Male Weight at PND24+	119 ± 21 (31-33 days)	109 ± 15 (29-33 days)	110 ± 19 (24-32 days)	119 ± 13 (27-33 days)	115 ± 19 (25-34 days)	123 ± 24 (24-34 days)	113 ± 25 (24-35 days)	124 ± 15 (31-35 days)
Female Weight at PND24+	101 ± 13 (31-33 days)	102 ± 13 (29-33 days)	101 ± 15 (24-32 days)	105 ± 16 (27-33 days)	109 ± 16 (25-34 days)	109 ± 18 (24-34 days)	98 ± 20 (24-35 days)	109 ± 12 (31-35 days)
Male Weight at PND60+	408 ± 52 (72-74 days)	423 ± 32 (70-74 days)	447 ± 50 (65-73 days)	405 ± 39 (69-73 days)	411 ± 36 (67-73 days)	410 ± 33 (60-73 days)	404 ± 35 (60-76 days)	428 ± 43 (61-74 days)
Female Weight at PND60+	254 ± 35 (72-74 days)	267 ± 18 (70-74 days)	266 ± 15 (65-73 days)	267 ± 22 (69-73 days)	241 ± 20 (67-73 days)	240 ± 10 (60-73 days)	236 ± 25 (60-76 days)	259 ± 29 (61-74 days)

See Table 2 for description of groups

Table 10: Weight Change Data (±SD) in P1 female rats during exposure period (Day 1 through Day 28); n=(x).

Endpoint	**Group 1** Control (56)	**Group 2** Low Dose (56)	**Group 3** Mid Dose (55)	**Group 4** High Dose (55)
Initial Weight (grams)	247 ± 13	252 ± 17	247 ± 17	248 ± 17
Final Weight (grams)	289 ± 24	300 ± 26	284 ± 25	291 ± 25
Weight Change (grams)	42 ± 17	48 ± 19	37 ± 15	43 ± 13
Percentage Weight Change	Δ = + 17.1 %	Δ = + 18.9 %	Δ = + 14.9 %	Δ = + 17.2 %

Table 11: Weight Change Data (±SD) in P1 male rats during exposure period (Day 1 through Day 28); n=28.

Endpoint	**Group 1** Control	**Group 2** Low Dose	**Group 3** Mid Dose	**Group 4** High Dose
Initial Weight (grams)	387 ± 17	390 ± 37	388 ± 18	385 ± 19
Final Weight (grams)	490 ± 37	488 ± 37	462 ± 32	474 ± 33
Weight Change (grams)	103 ± 24	98 ± 19	**74[Ŧ] ± 21**	89 ± 26
Percentage Weight Change	Δ = + 26.6 %	Δ = + 25.1 %	Δ = + 19.0 %	Δ = + 23.1 %

Ŧ One-way ANOVA indicated statistically significant differences in body weight increases between the mid dose group and controls.

Table 12: Mean organ weights in grams (±SD) for adult female rats (P) sacrificed 5-8 weeks following a continuous 28-day exposure, pregnancy, and weaning; n=16.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Left Kidney	1.15 ± 0.11	1.17 ± 0.12	1.07 ± 0.12	1.20 ± 0.12
Right kidney	1.19 ± 0.11	1.21 ± 0.14	1.11 ± 0.21	1.21 ± 0.11
Spleen	0.61 ± 0.08	0.62 ± 0.08	0.61 ± 0.11	0.59 ± 0.06
Liver	11.45 ± 0.98	10.64 ± 1.29	10.53 ± 0.21	11.64 ± 3.07
Ovary	0.92 ± 0.33	0.96 ± 0.30	1.00 ± 0.3	0.78 ± 0.17

Table 13: Mean organ weights in grams (±SD) for adult male rats (P) sacrificed 3-5 weeks following a continuous 28-day exposure; n=8 except where noted.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Left Kidney	1.85 ± 0.21	1.62 ± 0.14	1.46 ± 0.17	1.62 ± 0.19 (7)
Right kidney	1.831 ± 0.20	1.61 ± 0.14	1.60 ± 0.17	1.57 ± 0.24
Spleen	0.77 ± 0.12	0.77 ± 0.09	0.80 ± 0.11	0.80 ± 0.17
Liver	16.03 ± 2.08	13.81 ± 1.97	12.91 ± 1.16	13.09 ± 1.45
Testes	4.71 ± 0.35	4.83 ± 0.31	4.74 ± 0.44	4.57 ± 0.13

Table 14: Mean organ weights in grams (±SD) for female F1 rat pups (age 24-35 days), both of whose parents were exposed to continuous 28-day exposure; n=8.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Brain	1.60 ± 0.04	1.61 ± 0.07	1.61 ± 0.04	1.61 ± 0.07
Heart	0.61 ± 0.08	0.60 ± 0.05	0.65 ± 0.08	0.61 ± 0.07
Left Kidney	0.60 ± 0.07	0.58 ± 0.07	0.58 ± 0.06	0.58 ± 0.07
Right kidney	0.61 ± 0.07	0.60 ± 0.08	0.60 ± 0.08	0.59 ± 0.08
Spleen	0.36 ± 0.06	0.42 ± 0.05	0.38 ± 0.07	0.35 ± 0.07
Liver	4.07 ± 0.62	4.02 ± 0.50	4.14 ± 0.51	3.78 ± 0.42
Ovary	0.28 ± 0.13	**0.18Ŧ ± 0.09**	**0.19Ŧ ± 0.07**	**0.19Ŧ ± 0.10**

Ŧ ANCOVA indicated statistically significant differences (decreases) between the mean ovary weight of female offspring (age24-35 days) of dams and sires from the dose groups compared to controls.

Table 15: Mean organ weights in grams (±SD) for female F1 rat pups (age 24-35 days), whose mothers were exposed to continuous 28-day exposure, but whose fathers were unexposed; n=8.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Brain	1.57 ± 0.05	1.65 ± 0.11	1.64 ± 0.07	1.63 ± 0.16
Heart	0.65 ± 0.10	0.64 ± 0.08	0.67 ± 0.09	0.67 ± 0.06
Left Kidney	0.60 ± 0.04	0.62 ± 0.08	0.63 ± 0.08	0.64 ± 0.07
Right kidney	0.60 ± 0.04	0.60 ± 0.06	0.64 ± 0.09	0.65 ± 0.08
Spleen	0.40 ± 0.06	0.36 ± 0.05	0.38 ± 0.07	0.40 ± 0.06
Liver	4.02 ± 0.43	3.98 ± 0.28	4.14 ± 0.19	4.02 ± 0.40
Ovary	0.27 ± 0.13	**0.14Ŧ ± 0.04**	**0.21Ŧ ± 0.08**	**0.21Ŧ ± 0.09**

Ŧ ANCOVA indicated statistically significant differences (decreases) between the mean ovary weight of female offspring (age24-35 days) of dams from the dose groups compared to controls.

Table 16: Mean organ weights in grams (±SD) for male F1 rat pups (24-35 days old), both of whose parents were exposed to continuous 28-day exposure; n=8.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Brain	1.67 ± 0.06	1.68 ± 0.08	1.66 ± 0.11	1.71 ± 0.11
Heart	0.71 ± 0.08	0.62 ± 0.10	0.69 ± 0.09	0.70 ± 0.11
Left Kidney	0.68 ± 0.08	0.64 ± 0.12	0.64 ± 0.07	0.65 ± 0.12
Right kidney	0.72 ± 0.09	0.66 ± 0.13	0.67 ± 0.06	0.70 ± 0.13
Spleen	0.43 ± 0.10	0.45 ± 0.06	0.49 ± 0.07	0.45 ± 0.11
Liver	4.64 ± 0.92	4.41 ± 0.73	4.52 ± 0.76	4.32 ± 0.51
Testes	1.05 ± 0.19	0.93 ± 0.24	0.99 ± 0.20	1.03 ± 0.35

Table 17: Mean organ weights in grams (±SD) for male F1 rat pups (24-35 days old), whose mothers were exposed to continuous 28-day exposure, but whose fathers were unexposed; n=8.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Brain	1.63 ± 0.07	1.74 ± 0.05	1.73 ± 0.07	1.70 ± 0.07
Heart	0.65 ± 0.05	0.77 ± 0.14	0.69 ± 0.09	0.72 ± 0.05
Left Kidney	0.66 ± 0.07	0.71 ± 0.11	0.70 ± 0.12	0.72 ± 0.08
Right kidney	0.66 ± 0.07	0.71 ± 0.10	0.70 ± 0.12	0.75 ± 0.09
Spleen	0.46 ± 0.06	0.45 ± 0.10	0.48 ± 0.11	0.46 ± 0.07
Liver	4.18 ± 0.40	4.52 ± 0.54	4.62 ± 0.83	4.52 ± 0.43
Testes	1.01 ± 0.20	1.12 ± 0.18	1.09 ± 0.34	1.17 ± 0.20

Table 18: Mean organ weights in grams (±SD) for adult female F1 rats (60-76 days old), both of whose parents were exposed to continuous 28-day exposure; n=8.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Brain	1.87 ± 0.12	1.95 ± 0.07	1.89 ± 0.10	1.88 ± 0.07
Heart	1.24 ± 0.17	**1.15Ŧ ± 0.08**	**1.05Ŧ ± 0.09**	**1.05Ŧ ± 0.09**
Left Kidney	1.07 ± 0.17	1.11 ± 0.06	0.96 ± 0.09	0.99 ± 0.09
Right kidney	1.10 ± 0.15	1.12 ± 0.06	0.88 ± 0.30	1.01 ± 0.10
Spleen	0.55 ± 0.09	0.61 ± 0.11	0.55 ± 0.06	0.52 ± 0.05
Liver	8.10 ± 1.10	10.14 ± 1.56	7.92 ± 0.67	7.83 ± 0.69
Ovary	0.67 ± 0.23	0.71 ± 0.22	0.61 ± 0.17	0.63 ± 0.20

Ŧ ANCOVA indicated statistically significant differences (decreases) between the mean heart weight of female offspring (age60-76 days) of dams and sires from the dose groups compared to controls.

Table 19: Mean organ weights in grams (±SD) for adult female F1 rats (60-74 days old), whose mothers were exposed to continuous 28-day exposure, but whose fathers were unexposed; n=8.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Brain	1.77 ± 0.32	1.94 ± 0.10	1.88 ± 0.09	1.91 ± 0.07
Heart	1.26 ± 0.15	**1.09Ŧ ± 0.10**	**1.05Ŧ ± 0.08**	**1.13Ŧ ± 0.09**
Left Kidney	1.11 ± 0.08	1.09 ± 0.14	1.09 ± 0.24	1.07 ± 0.09
Right kidney	1.14 ± 0.13	1.08 ± 0.15	1.11 ± 0.23	1.12 ± 0.10
Spleen	0.60 ± 0.09	0.57 ± 0.06	0.53 ± 0.08	0.54 ± 0.69
Liver	8.70 ± 0.63	9.64 ± 0.62	7.71 ± 0.57	8.51 ± 1.05
Ovary	0.74 ± 0.18	0.76 ± 0.25	0.58 ± 0.06	0.59 ± 0.06

Ŧ ANCOVA indicated statistically significant differences (decreases) between the mean heart weight of female offspring (age60-76 days) of dams from the dose groups compared to controls.

Table 20: Mean organ weights in grams (±SD) for adult male F1 rats (60-76 days old), both of whose parents were exposed to continuous 28-day exposure; n=8.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Brain	1.99 ± 0.13	2.14 ± 0.09	1.97 ± 0.20	2.03 ± 0.09
Heart	1.63 ± 0.21	1.75 ± 0.15	1.82 ± 0.24	1.64 ± 0.18
Left Kidney	1.70 ± 0.24	1.81 ± 0.19	1.58 ± 0.16	1.66 ± 0.15
Right kidney	1.73 ± 0.24	1.90 ± 0.23	1.59 ± 0.17	1.64 ± 0.19
Spleen	0.69 ± 0.09	0.89 ± 0.13	0.77 ± 0.11	0.82 ± 0.11
Liver	13.26 ± 2.4	18.74 ± 3.30	13.29 ± 1.64	13.66 ± 2.06
Testes	4.10 ± 0.32	4.40 ± 0.37	4.39 ± 0.27	4.43 ± 0.46

Table 21: Mean organ weights in grams (±SD) for adult male F1 rats (60-74 days old), whose mothers were exposed to continuous 28-day exposure, but whose fathers were unexposed; n=8.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Brain	1.99 ± 0.09	2.06 ± 0.07	2.06 ± 0.08	2.08 ± 0.10
Heart	1.70 ± 0.15	1.61 ± 0.13	1.50 ± 0.13	1.70 ± 0.27
Left Kidney	1.70 ± 0.21	1.69 ± 0.12	1.66 ± 0.12	1.73 ± 0.24
Right kidney	1.70 ± 0.19	1.72 ± 0.10	1.70 ± 0.11	1.70 ± 0.20
Spleen	0.83 ± 0.09	0.84 ± 0.08	0.79 ± 0.09	0.81 ± 0.11
Liver	14.49 ± 1.75	14.72 ± 2.49	12.58 ± 1.27	13.29 ± 1.53
Testes	4.51 ± 0.33	4.45 ± 0.21	4.43 ± 0.43	4.41 ± 0.27

Table 22: Hematology values (±SD) measured in adult female rats 5-8 weeks after a continuous 28-day exposure; n=(x). The standard reference ranges for CD® IGS rats female rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
WBC ($5.5 – 11.7 \times 10^3/\mu L$)	11.0 ± 3.1 (16)	9.9 ± 2.3 (16)	10.4 ± 2.0 (15)	10.3 ± 2.5 (16)
Lymphocytes ($3.3 – 9.5 \times 10^3/\mu L$)	5.6 ± 1.5 (15)	4.8 ± 1.5 (16)	5.1 ± 1.3 (14)	5.0 ± 1.3 (15)
% Lymphocytes	54 ± 5.9 (16)	49 ± 7.7 (16)	51 ± 8.9 (15)	51 ± 7.5 (16)
Monocytes ($0.10 – 0.90 \times 10^3/\mu L$)	1.0 ± 0.3 (15)	0.96 ± 0.31 (15)	1.0 ± 0.27 (15)	0.98 ± 0.30 (15)
% Monocytes	10 ± 2.7 (16)	10 ± 3.0 (16)	10 ± 1.8 (15)	9.6 ± 1.6 (15)
Neutrophils ($0.5 – 8.1 \times 10^3/\mu L$)	3.7 ± 1.1 (16)	3.8 ± 0.9 (16)	3.8 ± 0.8 (15)	3.9 ± 1.1 (16)
% Neutrophils	34 ± 5.1 (16)	39 ± 7.1 (16)	37 ± 6.8 (15)	38 ± 6.2 (16)
Eosinophils ($0.10 – 0.90 \times 10^3/\mu L$)	0.21 ± 0.20 (16)	0.17 ± 0.16 (16)	0.15 ± 0.20 (15)	0.10 ± 0.06 (15)
% Eosinophils	1.9 ± 1.5 (16)	1.7 ± 1.3 (16)	1.1 ± 1.4 (15)	1.0 ± 0.5 (15)
Basophils ($\leq 0.11 \times 10^3/\mu L$)	0.05 ± 0.12 (16)	0.05 ± 0.08 (16)	0.03 ± 0.08 (15)	0.03 ± 0.07 (16)
% Basophils	0.43 ± 0.85 (16)	0.33 ± 0.49 (15)	0.30 ± 0.71 (15)	0.13 ± 0.17 (15)
RBC ($5.9 – 8.3 \times 10^6/\mu L$)	7.9 ± 0.30 (16)	8.0 ± 0.40 (16)	8.0 ± 0.46 (15)	8.0 ± 0.43 (15)
% RDW	17 ± 0.8 (16)	17 ± 0.6 (16)	17 ± 0.7 (15)	17 ± 0.9 (16)
HB ($8.6 – 15.7 \times 10^6/\mu L$)	16.1 ± 0.68 (16)	16.6 ± 0.71 (16)	16.5 ± 0.94 (15)	16.4 ± 0.78 (15)
% Hematocrit (10 – 55)	51 ± 2 (16)	53 ± 2 (16)	52 ± 3 (15)	52 ± 4 (16)
MCV ($54 – 69\ mm^3$)	65 ± 2 (15)	66 ± 2 (16)	65 ± 3 (15)	66 ± 2 (16)
MCH (18 – 22 pg)	20 ± 1 (16)	21 ± 1 (16)	21 + 1 (15)	21 ± 2 (16)
MCHC (29 – 36 g/dL)	31 ± 1 (16)	32 ± 1 (16)	32 ± 1 (15)	31 ± 2 (16)
PLT ($380 – 1210 \times 10^3/\mu L$)	1091 ± 179 (16)	1019 ± 194 (16)	981 ± 267 (15)	1011 ± 199 (16)

Table 23: Hematology values (±SD) measured in adult male rats 3-5 weeks after a continuous 28-day exposure; n=(x). The standard reference ranges for CD® IGS male rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
WBC ($3.9 – 17.2 \times 10^3/\mu L$)	11.0 ± 2.4 (7)	11.2 ± 5.6 (7)	9.2 ± 2.7 (11)	9.4 ± 2.2 (10)
Lymphocytes ($1.2 – 10.7 \times 10^3/\mu L$)	6.8 ± 1.9 (7)	6.3 ± 3.6 (7)	5.0 ± 1.4 (11)	5.0 ± 1.4 (10)
% Lymphocytes	62 ± 12 (7)	55 ± 10 (7)	56 ± 9 (11)	54 ± 8 (10)
Monocytes ($0.10 – 0.90 \times 10^3/\mu L$)	0.58 ± 0.21 (7)	0.57 ± 0.30 (6)	0.75 ± 0.28 (11)	0.82 ± 0.27 (10)
% Monocytes	5 ± 1.5 (7)	7 ± 3.5 (7)	8 ± 1.6 (11)	**9Ŧ ± 2.1** (10)
Neutrophils ($0.5 – 8.1 \times 10^3/\mu L$)	3.5 ± 1.3 (7)	4.0 ± 1.9 (7)	3.4 ± 1.4 (11)	3.5 ± 1.1 (10)
% Neutrophils	31 ± 10 (7)	37 ± 8 (7)	36 ± 9 (11)	37 ± 7 (10)
Eosinophils ($0.10 – 0.40 \times 10^3/\mu L$)	0.09 ± 0.06 (7)	0.10 ± 0.11 (7)	0.04 ± 0.05 (11)	0.02 ± 0.02 (10)
% Eosinophils	0.9 ± 0.6 (7)	0.5 ± 0.3 (6)	0.5 ± 0.5 (11)	0.3 ± 0.3 (10)
Basophils ($\leq 0.20 \times 10^3/\mu L$)	0.03 ± 0.03 (7)	0.02 ± 0.02 (7)	0.01 ± 0.01 (11)	0.00 ± 0.01 (10)
% Basophils	0.3 ± 0.3 (7)	0.1 ± 0.2 (7)	0.1 ± 0.2 (11)	0.1 ± 0.1 (10)
RBC ($5.1 – 9.0 \times 10^6/\mu L$)	7.5 ± 1.0 (7)	8.0 ± 0.6 (7)	8.8 ± 2.1 (11)	8.3 ± 0.4 (9)
% RDW	16.6 ± 0.5 (7)	17.0 ± 0.7 (7)	17.6 ± 1.9 (11)	17.2 ± 0.6 (10)
HB ($8.6 – 16.3 \times 10^6/\mu L$)	13.8 ± 1.8 (7)	14.7 ± 0.7 (7)	15.2 ± 1.3 (11)	16.0 ± 1.0 (7)
% Hematocrit (28 – 55)	43 ± 6 (7)	45 ± 2 (7)	50 ± 13 (11)	48 ± 3 (9)
MCV ($51 – 73\ mm^3$)	58 ± 1 (7)	57 ± 2 (7)	56 ± 2 (11)	58 ± 3 (10)
MCH (18 – 21 pg)	18 ± 1 (7)	18 ± 1 (7)	8 ± 3 (11)	18 ± 3 (10)
MCHC (29 – 37 g/dL)	32 ± 1 (7)	32 ± 1 (7)	32 ± 6 (11)	31 ± 6 (10)
PLT ($380 – 1210 \times 10^3/\mu L$)	929 ± 272 (7)	930 ± 409 (7)	1298 ± 358 (11)	1024 ± 345 (10)

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Table 24: Hematology values (±SD) measured in female rat pups (age 24-35 days), whose parents were exposed to continuous 28-day exposure; n=(x). The standard reference ranges for CD® IGS female rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
WBC ($5.5 – 11.7 \times 10^3/\mu L$)	6.3 ± 2.5 (16)	8.3 ± 2.0 (16)	**8.7Ŧ ± 2.1** (17)	**8.7Ŧ ± 2.9** (16)
Lymphocytes ($3.3 – 9.5 \times 10^3/\mu L$)	3.7 ± 1.5 (16)	4.8 ± 1.4 (16)	5.0 ± 1.3 (17)	5.0 ± 2.2 (16)
% Lymphocytes	59 ± 7 (15)	58 ± 8 (16)	58 ± 6 (17)	57 ± 8 (16)
Monocytes ($0.10 – 0.90 \times 10^3/\mu L$)	0.37 ± 0.22 (15)	0.66 ± 0.14 (14)	**0.69Ŧ ± 0.39** (17)	**0.78Ŧ ± 0.38** (16)
% Monocytes	6 ± 3 (15)	9 ± 2 (16)	8 ± 3 (17)	9 ± 4 (16)
Neutrophils ($0.5 – 8.1 \times 10^3/\mu L$)	2.1 ± 1.0 (16)	2.6 ± 0.9 (16)	**2.9Ŧ ± 0.8** (17)	2.7 ± 0.8 (16)
% Neutrophils	32 ± 6 (15)	32 ± 8 (16)	33 ± 4 (17)	32 ± 8 (16)
Eosinophils ($0.10 – 0.90 \times 10^3/\mu L$)	0.09 ± 0.11 (15)	0.05 ± 0.07 (16)	0.07 ± 0.06 (16)	0.10 ± 0.07 (15)
% Eosinophils	1.1 ± 0.9 (15)	**0.4Ψ ± 0.4** (14)	0.8 ± 0.6 (16)	**1.7Ψ ± 1.6** (16)
Basophils ($\leq 0.11 \times 10^3/\mu L$)	0.03 ± 0.04 (16)	0.01 ± 0.02 (16)	0.03 ± 0.04 (17)	0.03 ± 0.02 (14)
% Basophils	0.4 ± 0.5 (16)	0.1 ± 0.2 (16)	0.3 ± 0.4 (16)	0.4 ± 0.3 (14)
RBC ($5.9 – 8.3 \times 10^6/\mu L$)	5.5 ± 0.7 (16)	5.5 ± 0.4 (16)	5.8 ± 0.6 (16)	**6.0Ψ ± 0.4** (15)
% RDW	26.3 ± 2.9 (16)	26.9 ± 3.3 (15)	26.6 ± 4.9 (16)	27.1 ± 4.8 (16)
HB ($8.6 – 15.7 \times 10^6/\mu L$)	11.1 ± 0.9 (15)	11.1 ± 0.8 (16)	11.6 ± 1.7 (16)	**12.2Ψ ± 2.1** (16)
% Hematocrit (10 – 55)	37 ± 4 (15)	37 ± 2 (15)	39 ± 5 (16)	40 ± 4 (16)
MCV ($54 – 69\ mm^3$)	67 ± 2 (15)	67 ± 2 (15)	68 ± 4 (16)	68 ± 4 (16)
MCH (18 – 22 pg)	20 ± 1 (15)	20 ± 1 (16)	21 + 1 (16)	**22Ŧ ± 1** (15)
MCHC (29 – 36 g/dL)	30 ± 1 (15)	31 ± 2 (16)	30 ± 1 (16)	**32Ŧ ± 1** (15)
PLT ($380 – 1210 \times 10^3/\mu L$)	666 ± 521 (16)	**1140Ψ ± 440** (16)	**1366Ψ ± 454** (16)	**1346Ψ ± 569** (16)

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

Table 25: Hematology values (±SD) measured in male rat pups (age 24-35 days), whose parents were exposed to continuous 28-day exposure; n=(x). The standard reference ranges for CD® IGS male rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
WBC ($3.9 – 17.2 \times 10^3/\mu L$)	6.7 ± 2.8 (14)	7.5 ± 2.5 (16)	8.6 ± 3.4 (15)	8.4 ± 1.9 (15)
Lymphocytes ($1.2 – 10.7 \times 10^3/\mu L$)	3.9 ± 1.5 (14)	4.3 ± 1.6 (16)	4.9 ± 1.9 (15)	4.8 ± 1.3 (16)
% Lymphocytes	60 ± 7 (15)	57 ± 6 (16)	57 ± 6 (15)	55 ± 5 (16)
Monocytes ($0.10 – 0.90 \times 10^3/\mu L$)	0.48 ± 0.29 (15)	0.60 ± 0.31 (16)	0.75 ± 0.53 (15)	**1.0Ŧ ± 0.55** (16)
% Monocytes	6 ± 2 (14)	8 ± 4 (15)	8 ± 5 (15)	11 ± 5 (16)
Neutrophils ($0.5 – 8.1 \times 10^3/\mu L$)	2.2 ± 1.1 (15)	2.5 ± 0.9 (16)	2.9 ± 1.1 (15)	2.7 ± 0.7 (15)
% Neutrophils	31 ± 7 (15)	34 ± 6 (16)	33 ± 3 (14)	33 ± 5 (16)
Eosinophils ($0.10 – 0.40 \times 10^3/\mu L$)	0.07 ± 0.10 (14)	0.05 ± 0.05 (14)	0.08 ± 0.08 (15)	0.06 ± 0.05 (14)
% Eosinophils	1.0 ± 1.0 (14)	0.9 ± 0.9 (16)	0.8 ± 0.8 (14)	0.9 ± 0.7 (15)
Basophils ($\leq 0.20 \times 10^3/\mu L$)	0.04 ± 0.07 (15)	0.01 ± 0.02 (15)	0.02 ± 0.02 (14)	0.02 ± 0.02 (15)
% Basophils	0.4 ± 0.6 (15)	0.1 ± 0.2 (14)	0.2 ± 0.3 (14)	0.2 ± 0.3 (15)
RBC ($5.1 – 9.0 \times 10^6/\mu L$)	5.6 ± 0.5 (15)	5.5 ± 0.3 (16)	5.7 ± 0.6 (14)	5.7 ± 0.6 (16)
% RDW	26.3 ± 3.6 (15)	28.9 ± 4.4 (16)	27.8 ± 4.8 (15)	27.8 ± 5.5 (16)
HB ($8.6 – 16.3 \times 10^6/\mu L$)	10.9 ± 0.7 (15)	11.2 ± 0.8 (16)	**12.2Ŧ ± 1.8** (14)	**12.3Ŧ ± 1.4** (16)
% Hematocrit (28 – 55)	38.3 ± 3.8 (15)	37.4 ± 2.7 (15)	39.3 ± 5.2 (14)	39.3 ± 5.1 (16)
MCV ($51 – 73\ mm^3$)	68 ± 4 (15)	68 ± 4 (16)	68 ± 4 (15)	70 ± 3 (15)
MCH (18 – 21 pg)	20 ± 2 (15)	20 ± 1 (16)	**21Ŧ ± 1** (15)	**22Ŧ ± 2** (15)
MCHC (29 – 37 g/dL)	29 ± 2 (14)	30 ± 1 (16)	**31Ŧ ± 2** (15)	**32Ŧ ± 1** (15)
PLT ($380 – 1210 \times 10^3/\mu L$)	995 ± 496 (14)	**1372Ψ ± 162** (15)	**1489Ψ ± 266** (14)	**1489Ψ ± 192** (15)

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

Table 26: Hematology values (±SD) measured in adult female rats (age 60-74 days), whose parents were exposed to continuous 28-day exposure; n=(x). The standard reference ranges for CD® IGS female rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
WBC (5.5 – 11.7 x 10^3/µL)	4.8 ± 3.2 (11)	**12.4Ŧ ± 3.6** (17)	**12.3Ŧ ± 3.8** (16)	**11.5Ŧ ± 2.7** (14)
Lymphocytes (3.3 – 9.5 x 10^3/µL)	3.3 ± 2.1 (11)	**7.4Ŧ ± 2.3** (17)	**7.3Ŧ ± 2.4** (16)	**6.5Ŧ ± 2.1** (14)
% Lymphocytes	70 ± 6 (11)	**60Ŧ ± 7** (17)	**60Ŧ ± 6** (15)	**54Ŧ ± 6** (13)
Monocytes (0.10 – 0.90 x 10^3/µL)	0.31 ± 0.37 (11)	**1.1Ŧ ± 0.44** (17)	**1.2Ŧ ± 0.63** (16)	**0.95Ŧ ± 0.41** (14)
% Monocytes	5 ± 3 (11)	**9Ŧ ± 3** (17)	**11Ŧ ± 5** (16)	**8Ŧ ± 3** (14)
Neutrophils (0.5 – 8.1 x 10^3/µL)	1.1 ± 0.7 (11)	**3.3Ŧ ± 0.8** (15)	**3.6Ŧ ± 1.1** (16)	**3.9Ŧ ± 1.0** (14)
% Neutrophils	23 ± 4 (11)	**29Ŧ ± 6** (17)	**30Ŧ ± 4** (15)	**34Ŧ ± 7** (14)
Eosinophils (0.10 – 0.90 x 10^3/µL)	0.04 ± 0.04 (10)	**0.16Ŧ ± 0.10** (16)	**0.12Ŧ ± 0.10** (16)	**0.15Ŧ ± 0.10** (14)
% Eosinophils	1.2 ± 1.1 (11)	1.6 ± 1.3 (17)	0.9 ± 0.6 (16)	1.3 ± 0.9 (14)
Basophils (≤ 0.11 x 10^3/µL)	0.02 ± 0.02 (10)	0.04 ± 0.05 (16)	0.03 ± 0.05 (16)	0.04 ± 0.04 (14)
% Basophils	0.4 ± 0.3 (10)	0.5 ± 0.8 (17)	0.2 ± 0.4 (16)	0.3 ± 0.3 (14)
RBC (5.9 – 8.3 x 10^6/µL)	4.2 ± 1.7 (11)	**7.2Ψ ± 0.8** (17)	**7.6Ψ ± 0.5** (15)	**7.5Ψ ± 0.9** (14)
% RDW	13.6 ± 1.0 (10)	**14.7Ψ ± 0.5** (17)	14.2 ± 0.6 (16)	**14.6Ψ ± 0.6** (14)
HB (8.6 – 15.7 x 10^6/µL)	8.0 ± 3.5 (11)	**14.9Ψ ± 1.5** (16)	**16.3Ψ ± 1.2** (15)	**15.9Ψ ± 2.1** (14)
% Hematocrit (10 – 55)	28 ± 12 (11)	**48Ψ ± 6** (17)	**51Ψ ± 4** (15)	**50Ψ ± 6** (14)
MCV (54 – 69 mm^3)	67 ± 3 (10)	67 ± 2 (17)	67 ± 2 (16)	67 ± 2 (14)
MCH (18 – 22 pg)	19 ± 2 (11)	**21Ψ ± 1** (16)	**22Ψ + 1** (16)	**21Ψ ± 1** (14)
MCHC (29 –36 g/dL)	28 ± 2 (10)	**31Ψ ± 1** (16)	**32Ψ ± 1** (16)	**32Ψ ± 1** (14)
PLT (380 – 1210 x 10^3/µL)	140 ± 101 (10)	**856Ψ ± 391** (17)	**846Ψ ± 404** (16)	**767Ψ ± 348** (14)

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

Table 27: Hematology values (±SD) measured in adult male rats (age 60-74 days), whose parents were exposed to continuous 28-day exposure; n=(x). The standard reference ranges for CD® IGS male rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
WBC ($3.9 – 17.2 \times 10^3/\mu L$)	9.6 ± 4.8 (16)	**13.2$^{\Psi}$ ± 2.7** (16)	10.7 ± 5.5 (14)	**14.1$^{\Psi}$ ± 2.4** (17)
Lymphocytes ($1.2 – 10.7 \times 10^3/\mu L$)	5.8 ± 2.5 (16)	7.6 ± 1.9 (16)	5.8 ± 3.3 (14)	7.9 ± 1.0 (16)
% Lymphocytes	62 ± 7 (16)	59 ± 4 (15)	**53$^{\text{Ŧ}}$ ± 10** (14)	**55$^{\text{Ŧ}}$ ± 5** (17)
Monocytes ($0.10 – 0.90 \times 10^3/\mu L$)	0.75 ± 0.72 (16)	**1.5$^{\Psi}$ ± 0.52** (16)	1.2 ± 0.80 (14)	**1.4$^{\Psi}$ ± 0.30** (17)
% Monocytes	6 ± 4 (16)	**11$^{\Psi}$ ± 4** (16)	**11$^{\Psi}$ ± 4** (14)	**10$^{\Psi}$ ± 2** (18)
Neutrophils ($0.5 – 8.1 \times 10^3/\mu L$)	2.9 ± 1.6 (16)	4.0 ± 1.0 (16)	3.6 ± 1.9 (14)	**4.9$^{\Psi}$ ± 1.3** (18)
% Neutrophils	30 ± 5 (16)	30 ± 5 (16)	34 ± 8 (14)	33 ± 4 (17)
Eosinophils ($0.10 – 0.40 \times 10^3/\mu L$)	0.10 ± 0.07 (16)	0.07 ± 0.07 (15)	0.09 ± 0.08 (14)	0.18 ± 0.18 (17)
% Eosinophils	1.1 ± 0.6 (16)	**0.6$^{\Psi}$ ± 0.9** (15)	1.4 ± 2.4 (13)	1.2 ± 1.1 (17)
Basophils ($\leq 0.20 \times 10^3/\mu L$)	0.03 ± 0.03 (15)	**0.01$^{\Psi}$ ± 0.02** (16)	**0.02$^{\Psi}$ ± 0.03** (14)	0.03 ± 0.03 (17)
% Basophils	0.4 ± 0.3 (15)	0.1 ± 0.2 (16)	0.2 ± 0.3 (14)	0.2 ± 0.2 (17)
RBC ($5.1 – 9.0 \times 10^6/\mu L$)	6.4 ± 1.6 (16)	7.5 ± 0.4 (16)	6.5 ± 2.8 (13)	**7.8$^{\Psi}$ ± 0.5** (18)
% RDW	15.8 ± 1.2 (15)	16.0 ± 1.0 (16)	16.8 ± 6.3 (13)	15.8 ± 0.7 (17)
HB ($8.6 – 16.3 \times 10^6/\mu L$)	12.9 ± 3.6 (16)	16.0 ± 0.5 (15)	**16.2$^{\Psi}$ ± 2.02** (13)	**17.0$^{\Psi}$ ± 1.0** (18)
% Hematocrit (28 – 55)	43 ± 11 (16)	51 ± 2 (15)	44 ± 19 (13)	**53$^{\Psi}$ ± 4** (18)
MCV ($51 – 73\ mm^3$)	67 ± 2 (15)	67 ± 3 (16)	67 ± 6 (14)	68 ± 3 (18)
MCH (18 – 21 pg)	20 ± 1 (16)	21 ± 1 (16)	**22$^{\Psi}$ + 1** (12)	**22$^{\Psi}$ ± 1** (18)
MCHC (29 – 37 g/dL)	29 ± 1 (16)	**31$^{\Psi}$ ± 1** (16)	**31$^{\Psi}$ ± 9** (12)	**32$^{\Psi}$ ± 1** (18)
PLT ($380 – 1210 \times 10^3/\mu L$)	579 ± 486 (16)	**1127$^{\Psi}$ ± 151** (15)	984 ± 342 (13)	**1109$^{\Psi}$ ± 192** (17)

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

Table 28: Serum chemistries (±SD) measured in adult female rats sacrificed 5-8 weeks after a continuous 28-day exposure; n=(x). The standard reference ranges for CD® IGS female rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
TP (5.7 – 8.9 g/dL)	6.6 ± 0.4 (14)	6.9 ± 0.4 (16)	6.7 ± 0.4 (16)	6.6 ± 0.3 (16)
ALB (3.3 – 6.7 g/dL)	3.7 ± 0.3 (14)	4.0 ± 0.4 (16)	3.8 ± 0.3 (16)	3.7 ± 0.2 (16)
ALKP (90 – 205 U/L)	124 ± 76 (14)	88 ± 36 (16)	109 ± 62 (16)	122 ± 55 (16)
ALT (23 – 186 U/L)	54 ± 11 (14)	55 ± 21 (16)	60 ± 19 (16)	55 ± 10 (16)
AST (78 – 226 U/L)	68 ± 12 (13)	86 ± 27 (16)	83 ± 26 (15)	67 ± 10 (16)
BUN (10 – 25 mg/dL)	17 ± 4 (14)	19 ± 3 (16)	19 ± 3 (16)	19 ± 3 (16)
CHOL (47 – 92 mg/dL)	80 ± 13 (14)	83 ± 18 (16)	80 ± 13 (16)	90 ± 18 (16)
CK (117 – 531 U/L)	92 ± 33 (14)	117 ± 46 (16)	84 ± 29 (15)	90 ± 32 (16)
CREA (0.50 – 0.90 mg/dL)	0.46 ± 0.05 (14)	0.61 ± 0.13 (16)	0.52 ± 0.07 (16)	0.48 ± 0.04 (16)
GLU (81 – 185 mg/dL)	154 ± 15 (14)	178 ± 28 (16)	169 ± 23 (16)	160 ± 17 (16)
TBIL (0.10 – 1.00 mg/dL)	0.2 ± 0.07 (14)	0.26 ± 0.14 (16)	0.23 ± 0.09 (16)	0.21 ± 0.07 (16)
TRIG (30 – 205 mg/dL)	126 ± 43 (14)	74 ± 18 (16)	112 ± 64 (16)	105 ± 44 (16)
Na+ (140 – 156 mEq/L)	152 ± 2 (14)	162 ± 13 (16)	152 ± 4 (16)	151 ± 2 (16)
K+ (4.1 – 6.9 mEq/L)	6.3 ± 03 (13)	7.5 ± 0.7 (16)	6.6 ± 0.5 (15)	6.7 ± 0.5 (16)
Cl- (95 – 111 mEq/L)	104 ± 2 (14)	110 ± 7 (16)	104 ± 3 (16)	102 ± 3 (16)

Table 29: Serum chemistries (±SD) measured in adult male rats sacrificed 3-5 weeks after a continuous 28-day exposure; n=(x). The standard reference ranges for CD® IGS male rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
TP (5.6 – 8.1 g/dL)	6.2 ± 0.2 (9)	5.9 ± 0.3 (10)	6.1 ± 0.4 (12)	6.0 ± 0.4 (8)
ALB (3.2 – 5.2 g/dL)	3.5 ± 0.3 (9)	3.2 ± 0.3 (10)	3.3 ± 0.3 (12)	3.3 ± 0.3 (8)
ALKP (136 – 268 U/L)	202 ± 45 (9)	188 ± 55 (10)	168 ± 53 (12)	185 ± 41 (8)
ALT (27 – 97 U/L)	59 ± 15 (9)	47 ± 5 (9)	42 ± 9 (12)	42 ± 6 (8)
AST (77 – 246 U/L)	87 ± 22 (8)	83 ± 11 (9)	67 ± 14 (12)	72 ± 19 (8)
BUN (10 – 22 mg/dL)	19 ± 3 (9)	18 ± 3 (10)	16 ± 3 (12)	**15[Ŧ] ± 3** (8)
CHOL (24 – 92 mg/dL)	47 ± 9 (9)	47 ± 13 (10)	51 ± 15 (12)	38 ± 13 (8)
CK (56 – 477 U/L)	126 ± 54 (8)	129 ± 63 (9)	77 ± 20 (11)	104 ± 26 (7)
CREA (0.40 – 0.80 mg/dL)	0.50 ± 0.01 (9)	0.50 ± 0.05 (10)	0.49 ± 0.05 (12)	0.51 ± 0.04 (7)
GLU (85 – 197 mg/dL)	184 ± 37 (8)	192 ± 56 (10)	158 ± 11 (11)	160 ± 17 (7)
TBIL (0.10 – 1.00 mg/dL)	0.19 ± 0.12 (9)	0.20 ± 0.09 (9)	0.15 ± 0.12 (11)	0.19 ± 0.11 (8)
TRIG (46 – 208 mg/dL)	94 ± 17 (8)	73 ± 31 (9)	68 ± 25 (12)	71 ± 23 (8)
Na+ (141 – 157 mEq/L)	154 ± 2 (9)	152 ± 2 (10)	155 ± 3 (12)	155 ± 4 (8)
K+ (4.7 – 7.3 mEq/L)	6.2 ± 0.2 (8)	6.4 ± 0.6 (9)	6.6 ± 0.9 (12)	6.4 ± 0.4 (7)
Cl- (97 – 110 mEq/L)	101 ± 2 (9)	103 ± 2 (10)	101 ± 2 (12)	103 ± 3 (8)

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Table 30: Serum chemistries (±SD) measured in female rat pups (age 24-35 days), whose parents were exposed to continuous 28-day exposure; n=(x). The standard reference ranges for CD® IGS female rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
TP (5.7 – 8.9 g/dL)	5.1 ± 0.3 (14)	4.8 ± 0.2 (16)	5.3 ± 0.2 (12)	5.1 ± 0.2 (13)
ALB (3.3 – 6.7 g/dL)	3.0 ± 0.2 (14)	2.8 ± 0.1 (16)	3.0 ± 0.16 (12)	3.0 ± 0.2 (13)
ALKP (90 – 205 U/L)	369 ± 48 (14)	395 ± 53 (16)	359 ± 63 (12)	416 ± 99 (14)
ALT (23 – 186 U/L)	47 ± 10 (14)	43 ± 6 (16)	44 ± 6 (12)	48 ± 9 (14)
AST (78 – 226 U/L)	120 ± 21 (13)	**97$^{\Psi}$ ± 9** (15)	**96$^{\Psi}$ ± 10** (12)	**99$^{\Psi}$ ± 23** (14)
BUN (10 – 25 mg/dL)	15 ± 5 (14)	14 ± 4 (16)	13 ± 4 (12)	12 ± 4 (14)
CHOL (47 – 92 mg/dL)	85 ± 17 (14)	76 ± 9 (16)	86 ± 7 (12)	84 ± 10 (13)
CK (117 – 531 U/L)	347 ± 137 (13)	284 ± 38 (15)	294 ± 40 (11)	318 ± 74 (13)
CREA (0.50 – 0.90 mg/dL)	0.36 ± 0.05 (14)	0.39 ± 0.06 (16)	0.31 ± 0.07 (12)	0.38 ± 0.08 (13)
GLU (81 – 185 mg/dL)	170 ± 24 (14)	163 ± 22 (16)	175 ± 22 (12)	**128$^{\text{Ŧ}}$ ± 17** (14)
TBIL (0.10 – 1.00 mg/dL)	0.31 ± 0.16 (13)	0.21 ± 0.13 (16)	0.17 ± 0.10 (12)	0.24 ± 0.14 (14)
TRIG (30 – 205 mg/dL)	69 ± 26 (14)	60 ± 23 (16)	61 ± 19 (12)	64 ± 16 (13)
Na+ (140 – 156 mEq/L)	146 ± 1 (13)	147 ± 2 (16)	145 ± 2 (12)	146 ± 1 (13)
K+ (4.1 – 6.9 mEq/L)	7.4 ± 0.7 (12)	7.2 ± 0.4 (15)	7.1 ± 0.8 (12)	6.9 ± 0.5 (13)
Cl- (95 – 111 mEq/L)	106 ± 1 (13)	105 ± 2 (16)	106 ± 1 (12)	98 ± 29 (14)

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

Table 31: Serum chemistries (±SD) measured in male rat pups (age 24-35 days), whose parents were exposed to continuous 28-day exposure; n=(x). The standard reference ranges for CD® IGS male rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
TP (5.6 – 8.1 g/dL)	4.9 ± 0.4 (15)	4.8 ± 0.2 (16)	5.2 ± 0.3 (12)	5.4 ± 0.5 (13)
ALB (3.2 – 5.2 g/dL)	2.9 ± 0.3 (15)	2.7 ± 0.2 (16)	2.9 ± 0.2 (12)	3.2 ± 0.4 (13)
ALKP (136 – 268 U/L)	406 ± 72 (15)	421 ± 41 (16)	421 ± 69 (12)	441 ± 64 (13)
ALT (27 – 97 U/L)	47 ± 8 (15)	44 ± 6 (16)	49 ± 7 (12)	52 ± 9 (13)
AST (77 – 246 U/L)	125 ± 24 (15)	**105$^{\Psi}$ ± 9** (9)	**99$^{\Psi}$ ± 15** (11)	120 ± 24 (12)
BUN (10 – 22 mg/dL)	13 ± 4 (15)	11 ± 3 (16)	12 ± 3 (12)	11 ± 4 (13)
CHOL (24 – 92 mg/dL)	69 ± 12 (15)	75 ± 9 (16)	76 ± 10 (12)	**90^{Ŧ} ± 14** (13)
CK (56 – 477 U/L)	247 ± 59 (13)	206 ± 45 (16)	232 ± 60 (12)	230 ± 81 (12)
CREA (0.40 – 0.80 mg/dL)	0.37 ± 0.05 (15)	0.39 ± 0.06 (16)	0.32 ± 0.06 (12)	0.42 ± 0.09 (13)
GLU (85 – 197 mg/dL)	176 ± 35 (15)	155 ± 14 (16)	158 ± 18 (12)	**116^{Ŧ} ± 23** (13)
TBIL (0.10 – 1.00 mg/dL)	0.46 ± 0.39 (15)	**0.13$^{\Psi}$ ± 0.05** (16)	**0.14$^{\Psi}$ ± 0.05** (11)	0.36 ± 0.27 (13)
TRIG (46 – 208 mg/dL)	56 ± 22 (15)	58 ± 26 (16)	56 ± 15 (12)	71 ± 26 (13)
Na+ (141 – 157 mEq/L)	146 ± 2 (15)	146 ± 3 (16)	146 ± 2 (12)	145 ± 7 (13)
K+ (4.7 – 7.3 mEq/L)	7.2 ± 0.6 (14)	7.0 ± 0.4 (16)	7.2 ± 0.6 (12)	7.5 ± 0.8 (13)
Cl- (97 – 110 mEq/L)	105 ± 1 (15)	105 ± 2 (16)	105 ± 2 (12)	104 ± 4 (13)

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

Table 32: Serum chemistries (±SD) measured in adult female rats (age 60-74 days), whose parents were exposed to continuous 28-day exposure; n=(x). The standard reference ranges for CD® IGS female rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
TP (5.7 – 8.9 g/dL)	6.6 ± 0.4 (14)	6.4 ± 0.5 (15)	6.7 ± 0.3 (12)	6.6 ± 0.5 (14)
ALB (3.3 – 6.7 g/dL)	4.0 ± 0.4 (14)	3.8 ± 0.4 (15)	3.8 ± 0.3 (12)	4.1 ± 0.4 (15)
ALKP (90 – 205 U/L)	137 ± 61 (13)	**197$^{\Psi}$ ± 65** (15)	147 ± 44 (12)	154 ± 49 (15)
ALT (23 – 186 U/L)	49 ± 12 (13)	55 ± 11 (15)	54 ± 8 (12)	51 ± 12 (15)
AST (78 – 226 U/L)	102 ± 32 (14)	84 ± 11 (15)	94 ± 22 (12)	96 ± 20 (15)
BUN (10 – 25 mg/dL)	18 ± 3 (14)	18 ± 3 (15)	15 ± 3 (12)	16 ± 3 (15)
CHOL (47 – 92 mg/dL)	77 ± 10 (14)	83 ± 14 (15)	84 ± 9 (12)	81 ± 15 (8)
CK (117 – 531 U/L)	297 ± 283 (13)	161 ± 45 (15)	184 ± 75 (12)	233 ± 115 (15)
CREA (0.50 – 0.90 mg/dL)	0.52 ± 0.15 (14)	0.47 ± 0.07 (15)	**0.41$^{\Psi}$ ± 0.03** (12)	0.52 ± 0.08 (15)
GLU (81 – 185 mg/dL)	195 ± 39 (14)	195 ± 28 (15)	181 ± 42 (12)	198 ± 33 (15)
TBIL (0.10 – 1.00 mg/dL)	0.59 ± 0.35 (14)	0.25 ± 0.11 (15)	0.35 ± 0.13 (11)	0.42 ± 0.20 (15)
TRIG (30 – 205 mg/dL)	57 ± 16 (12)	**79$^{\Psi}$ ± 25** (14)	55 ± 13 (11)	**74$^{\Psi}$ ± 26** (15)
Na+ (140 – 156 mEq/L)	147 ± 5 (14)	149 ± 3 (14)	150 ± 2 (12)	148 ± 4 (15)
K+ (4.1 – 6.9 mEq/L)	8.1 ± 1.2 (14)	**7.4$^{\Psi}$ ± 0.8** (15)	**6.9$^{\Psi}$ ± 0.4** (12)	**6.9$^{\Psi}$ ± 0.4** (14)
Cl- (95 – 111 mEq/L)	103 ± 2 (14)	103 ± 2 (15)	102 ± 2 (12)	102 ± 3 (15)

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

Table 33: Serum chemistries (±SD) measured in adult male rats (age 60-74 days), whose parents were exposed to continuous 28-day exposure; n=(x). The standard reference ranges for CD® IGS male rats are indicated in endpoint column (Giknis, 2006).

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
TP (5.6 – 8.1 g/dL)	6.2 ± 0.3 (17)	6.0 ± 0.3 (15)	6.3 ± 0.2 (11)	6.3 ± 0.3 (14)
ALB (3.2 – 5.2 g/dL)	3.5 ± 0.2 (17)	3.3 ± 0.1 (14)	3.4 ± 0.2 (12)	3.6 ± 0.1 (13)
ALKP (136 – 268 U/L)	228 ± 75 (17)	265 ± 63 (15)	243 ± 36 (11)	251 ± 67 (14)
ALT (27 – 97 U/L)	52 ± 7 (17)	54 ± 9 (15)	50 ± 8 (11)	57 ± 8 (14)
AST (77 – 246 U/L)	97 ± 34 (17)	89 ± 14 (15)	93 ± 19 (12)	95 ± 12 (13)
BUN (10 – 22 mg/dL)	22 ± 2.6 (17)	**19Ŧ ± 2** (15)	**17Ŧ ± 3** (12)	**16Ŧ ± 2** (13)
CHOL (24 – 92 mg/dL)	65 ± 15 (17)	73 ± 14 (15)	60 ± 13 (12)	73 ± 15 (14)
CK (56 – 477 U/L)	182 ± 106 (15)	168 ± 66 (15)	180 ± 55 (11)	203 ± 91 (13)
CREA (0.40 – 0.80 mg/dL)	0.54 ± 0.09 (17)	**0.47Ψ ± 0.08** (15)	**0.46Ψ ± 0.07** (11)	0.57 ± 0.07 (14)
GLU (85 – 197 mg/dL)	179 ± 27 (17)	182 ± 28 (15)	184 ± 34 (12)	162 ± 26 (13)
TBIL (0.10 – 1.00 mg/dL)	0.29 ± 0.21 (16)	0.28 ± 0.13 (15)	0.26 ± 0.10 (11)	0.28 ± 0.09 (13)
TRIG (46 – 208 mg/dL)	106 ± 54 (17)	122 ± 67 (15)	91 ± 30 (12)	**67Ψ ± 19** (14)
Na+ (141 – 157 mEq/L)	150 ± 2 (17)	151 ± 3 (15)	151 ± 3 (12)	151 ± 4 (14)
K+ (4.7 – 7.3 mEq/L)	7.5 ± 0.9 (17)	6.8 ± 0.6 (15)	6.8 ± 0.9 (12)	7.1 ± 0.8 (14)
Cl- (97 – 110 mEq/L)	102 ± 2 (17)	103 ± 2 (15)	102 ± 2 (12)	101 ± 2 (14)

Ŧ One-way ANOVA indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Conover-Inman procedures.

Table 34: Histopathological lesions identified in female rats sacrificed 5-8 weeks after being exposed for 28 consecutive days.

Endpoint	Group 1	Group 2	Group 3	Group 4
Adrenal Glands	N=16	N=17	N=16	N=15
Number of animals within normal limits	16	17	16	15
Brain (Basal Ganglia; Cortex; Hippocampus)	N=16	N=17	N=16	N=15
Number of animals within normal limits	16	16	16	15
- Chronic Infiltrates	0 -	1 (1+)	0 -	0 -
Heart	N=16	N=17	N=16	N=15
Number of animals within normal limits	13	16	14	14
- Inflammation and/or Congestion	1 (1+++)	1 (1+)	0 -	1 (1++)
- Cardiomyopathy	2 (1+/1++)	0 -	2 (2+)	0 -
Kidneys	N=16	N=17	N=16	N=15
Number of animals within normal limits	4	4	**11**[Ŧ]	6
- Chronic Infiltrates	3 (3+)	3 (2+/1++)	0 -	0 -
- Progressive Nephropathy	4 (2+/2++)	2 (1+/1++)	3 (2+/1++)	3 (2+/1++)
- Fibrosis	**3**[Ψ] (2+/1++)	0 -	0 -	0 -
- Mineralization	7 (4+/3++)	10 (8+/2++)	3 (2+/1++)	9 (5+/4++)
Liver	N=16	N=17	N=16	N=15
Number of animals within normal limits	7	11	11	8
- Chronic Infiltrates	8 (6+/2++)	4 (4+)	4 (4+)	6 (5+/1++)
- Degenerative Changes	2 (1+/1++)	2 (2++)	1 (1+)	1 (1+)
Mammary Gland	N=13	N=13	N=15	N=14
Number of animals within normal limits	12	12	15	13
- Hyperkeratosis	1 (1+++)	0 -	0 -	0 -
- Adenoma	0	1 (1++)	0 -	1 (1+)
Ovaries and Oviducts	N=16	N=17	N=16	N=15
Number of animals within normal limits	16	17	16	15
Pancreas	N=15	N=17	N=16	N=15
Number of animals within normal limits	10	13	10	13
- Chronic Infiltrates	3 (2+/1+)	0 -	1 (1++)	0 (0+)
- Degenerative Changes	3	4	6	2

	(3++)	(4++)	(1+/5++)	(2++)
Pituitary Gland	N=16	N=17	N=16	N=15
Number of animals within normal limits	14	16	16	15
- Hypertrophy	2 (1+/1++)	1 (1+)	0 -	0 -
Spleen	N=16	N=17	N=16	N=15
Number of animals within normal limits	16	17	16	15
Uterus and Uterine Horns	N=16	N=17	N=16	N=15
Number of animals within normal limits	12	13	8	14
- Dilation	4 (1++/3+++)	4 (3++/1+++)	8 (4++/4+++)	1 (1++)

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Ŧ Pearson Chi-square test indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Fisher's Exact Test.

Ψ Pearson Chi-square test indicated statistically significant differences between the dose groups and controls, which were not validated by pair-wise comparisons using Fisher's Exact Test.

Table 35: Histopathological lesions identified in male rats sacrificed 3-5 weeks after being exposed for 28 consecutive days.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Adrenal Glands	N=9	N=8	N=11	N=8
Number of animals within normal limits	9	8	11	8
Brain (Basal Ganglia; Cortex; Hippocampus)	N=9	N=8	N=11	N=8
Number of animals within normal limits	9	8	11	7
- Chronic Inflammation	0 -	0 -	0 -	1 (1+)
- Adhesion and/or Dilation	0 -	0 -	0 -	1 (1++)
Epididymis	N=9	N=8	N=11	N=8
Number of animals within normal limits	9	8	11	8
Heart	N=9	N=8	N=11	N=8
Number of animals within normal limits	7	5	5	5
- Inflammation	0 -	2 (1+/1++)	3 (1+/2++)	0 -
- Cardiomyopathy	2 (1+/1++)	2 (1+/1++)	3 (3+)	3 (3+)
Kidneys	N=9	N=8	N=11	N=8
Number of animals within normal limits	6	5	8	6
- Chronic Infiltrates	0 -	2 (2+)	1 (1++)	0 -
- Progressive Nephropathy	3 (2+/1++)	1 (1++)	2 (1+/1++)	2 (2+)
Liver	N=9	N=8	N=11	N=8
Number of animals within normal limits	5	2	6	5
- Chronic Infiltrates	4 (4+)	6 (5+/1++)	5 (5+)	3 (3+)
- Degenerative Changes	0 -	0 -	0 -	1 (1+)
Pancreas	N=9	N=8	N=11	N=8
Number of animals within normal limits	7	8	11	6
- Chronic Infiltrates	1 (1+)	0 -	0 -	1 (1++)
- Degenerative Changes	1 (1+)	0 -	0 -	1 (1++)
Pituitary Gland	N=9	N=8	N=11	N=8
Number of animals within normal limits	9	7	9	7
- Hypertrophy	0 -	1 (1+)	2 (2+)	1 (1+)
Spleen	N=9	N=8	N=11	N=7
Number of animals within normal limits	9	8	11	7
Testes	N=9	N=8	N=11	N=8
Number of animals within normal limits	9	8	11	8

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Table 36: Histopathological lesions identified in unexposed F1 female rat pups (age 24-35 days), whose parents were exposed for 28 consecutive days.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Adrenal Glands	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Brain (Basal Ganglia; Cortex; Hippocampus)	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Heart	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Kidneys	N=8	N=8	N=8	N=8
Number of animals within normal limits	**3Ψ**	**2Ψ**	7	6
- Chronic Infiltrates	3 (3+)	3 (2+/1++)	1 (1+)	0 -
- Fibrosis	1 (1+)	2 (1+/1++)	0 -	0 -
- Hyperplasia, Tubular	0 -	1 (1+)	0 -	0 -
- Dilation, Pelvis	0 -	0 -	0 -	1 (1++)
- Cysts	4 (4++)	3 (3++)	0 -	1 (1++)
Liver	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Mammary Gland	N=5	N=8	N=5	N=8
Number of animals within normal limits	5	8	5	8
Ovaries and Oviducts	N=7	N=8	N=8	N=7
Number of animals within normal limits	7	8	8	7
Pancreas	N=8	N=7	N=8	N=8
Number of animals within normal limits	8	7	8	8
Pituitary Gland	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	7	8
- Mineralization	0 -	0 -	1 (1+)	0 -
Spleen	N=7	N=8	N=8	N=8
Number of animals within normal limits	7	8	8	8
Uterus and Uterine Horns	N=8	N=8	N=8	N=8
Number of animals within normal limits	7	8	8	7
- Dilation	1 (1+++)	0 -	0 -	1 (1++)

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Ψ Pearson Chi-square test indicated statistically significant differences between the dose groups and controls, which were not validated by pair-wise comparisons using Fisher's Exact Test.

Table 37: Histopathological lesions identified in unexposed F1 female rat pups (age 24-35 days), whose mothers were exposed for 28 consecutive days and whose fathers were unexposed.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Adrenal Glands	N=8	N=7	N=8	N=9
Number of animals within normal limits	8	7	8	9
Brain (Basal Ganglia; Cortex; Hippocampus)	N=8	N=7	N=8	N=9
Number of animals within normal limits	8	7	8	9
Heart	N=8	N=7	N=8	N=9
Number of animals within normal limits	8	7	8	9
Kidneys	N=8	N=7	N=8	N=9
Number of animals within normal limits	**1**$^{\Psi}$	5	5	7
- Chronic Infiltrates	2 (1+/1++)	1 (1++)	1 (1+)	1 (1++)
- Fibrosis	**3**$^{\Psi}$ (3+)	0 -	0 -	1 (1+)
- Basophilia, Tubular	0 -	0 -	1 (1+)	0 -
- Dilation, Pelvis	1 (1++)	0 -	0 -	0 -
- Cysts	6 (1+/5++)	1 (1++)	1 (1++)	2 (1+/1++)
Liver	N=8	N=7	N=8	N=9
Number of animals within normal limits	8	7	8	9
Mammary Gland	N=8	N=5	N=7	N=6
Number of animals within normal limits	8	5	7	6
Ovaries and Oviducts	N=8	N=7	N=8	N=9
Number of animals within normal limits	8	7	8	9
Pancreas	N=8	N=7	N=8	N=9
Number of animals within normal limits	8	7	8	9
Pituitary Gland	N=8	N=7	N=8	N=9
Number of animals within normal limits	8	7	8	9
Spleen	N=8	N=7	N=8	N=9
Number of animals within normal limits	8	7	8	9
Uterus and Uterine Horns	N=8	N=7	N=8	N=9
Number of animals within normal limits	7	7	6	9
- Dilation	1 (1++)	0 -	2 (2++)	0 -

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Ψ Pearson Chi-square test indicated statistically significant differences between the dose groups and controls, which were not validated by pair-wise comparisons using Fisher's Exact Test.

Table 38: Histopathological lesions identified in unexposed F1 male rat pups (age 24-35 days), whose parents were exposed for 28 consecutive days.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Adrenal Glands	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	7	8
- Fibrosis	0 -	0 -	1 (1+++)	0 -
- Mineralization	0 -	0 -	1 (1+++)	0 -
Brain – Basal Ganglia; Cortex; Hippocampus	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Epididymis	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Heart	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Kidneys	N=8	N=8	N=8	N=8
Number of animals within normal limits	4	7	3	5
- Chronic Infiltrates	2 (1+/1++)	1 (1+)	2 (1+/1++)	1 (1+)
- Fibrosis	0 -	0 -	0 -	1 (1+)
- Degeneration, Tubular	0 -	0 -	1 (1+)	0 -
- Dilation, Pelvis	1 (1+++)	0 -	0 -	1 (1++)
- Cysts	4 (2+/2++)	1 (1+)	4 (2+/2++)	1 (1++)
Liver	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Pancreas	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Pituitary Gland	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Spleen	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Testes	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Table 39: Histopathological lesions identified in unexposed F1 male rat pups (age 24-35 days), whose mothers were exposed for 28 consecutive days and whose fathers were unexposed.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Adrenal Glands	N=8	N=7	N=8	N=7
Number of animals within normal limits	8	7	8	7
Brain (Basal Ganglia; Cortex; Hippocampus)	N=8	N=7	N=8	N=7
Number of animals within normal limits	8	7	8	7
Epididymis	N=8	N=7	N=7	N=7
Number of animals within normal limits	8	6	7	7
- Infiltrates	0 -	1 (1++)	0 -	0 -
Heart	N=8	N=7	N=8	N=7
Number of animals within normal limits	8	7	8	7
Kidneys	N=8	N=7	N=8	N=7
Number of animals within normal limits	2	4	3	4
- Chronic Infiltrates	5 (3+/1++/1+++)	2 (2+)	1 (1+)	1 (1+)
- Basophilia, Tubular	0 -	0 -	1 (1+)	0 -
- Fibrosis	0 -	0 -	1 (1+)	0 -
- Dilation, Pelvis	2 (2++)	2 (1++/1+++)	1 (1++)	1 (1++)
- Cysts	4 (4+)	2 (2+)	3 (2+/1++)	3 (1+/2++)
Liver	N=8	N=7	N=8	N=7
Number of animals within normal limits	8	7	8	7
Pancreas	N=8	N=7	N=8	N=7
Number of animals within normal limits	8	7	8	7
Pituitary Gland	N=8	N=7	N=8	N=7
Number of animals within normal limits	8	7	8	7
Spleen	N=8	N=7	N=8	N=7
Number of animals within normal limits	8	7	8	7
Testes	N=8	N=7	N=7	N=7
Number of animals within normal limits	8	7	7	7

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Table 40: Histopathological lesions identified in unexposed F1 adult female rats (age 60-74 days), whose parents were exposed for 28 consecutive days.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Adrenal Glands	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Brain (Basal Ganglia; Cortex; Hippocampus)	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Heart	N=8	N=8	N=8	N=7
Number of animals within normal limits	7	8	8	7
- Cardiomyopathy	1 (1+)	0 -	0 -	0 -
Kidneys	N=8	N=8	N=8	N=8
Number of animals within normal limits	4	4	7	6
- Chronic Infiltrates	1 (1+)	1 (1+)	1 (1+)	0 -
- Progressive Nephropathy	4 (4+)	0 -	0 -	2 (2+)
- Mineralization	1 (1++)	3 (2+/1++)	0 -	1 (1+)
Liver	N=8	N=8	N=8	N=8
Number of animals within normal limits	6	6	3	6
- Chronic Infiltrates	2 (2+)	2 (2+)	4 (3+/1++)	2 (2+)
- Apoptosis	0 -	0 -	1 (1+)	0 -
- Vacuolation	0 -	0 -	1 (1+)	0 -
Mammary Gland	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Ovaries and Oviducts	N=7	N=8	N=8	N=7
Number of animals within normal limits	7	8	8	7
Pancreas	N=8	N=7	N=8	N=8
Number of animals within normal limits	8	6	7	8
- Chronic Infiltrates	0 -	1 (1+)	0 -	0 -
- Degenerative Changes	0 -	0 -	1 (1+)	0 -
Pituitary Gland	N=4	N=8	N=8	N=8
Number of animals within normal limits	4	8	8	8
Spleen	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Uterus and Uterine Horns	N=8	N=8	N=8	N=8
Number of animals within normal limits	7	7	7	5
- Dilation	1 (1++)	1 (1++)	1 (1++)	3 (3++)

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Table 41: Histopathological lesions identified in unexposed F1 adult female rats (age 60-74 days), whose mothers were exposed for 28 consecutive days and whose fathers were unexposed.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Adrenal Glands	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Brain (Basal Ganglia; Cortex; Hippocampus)	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Heart	N=8	N=8	N=8	N=7
Number of animals within normal limits	7	8	8	7
- Cardiomyopathy	1 (1+)	0 -	0 -	0 -
Kidneys	N=8	N=8	N=8	N=8
Number of animals within normal limits	4	5	4	5
- Chronic Infiltrates	2 (1+/1++)	1 (1+)	0 -	0 -
- Progressive Nephropathy	2 (1+/1++)	0 -	0 --	3 (2+/1++)
- Mineralization	1 (1+)	1 (1+)	4 (4+)	2 (2+)
- Dilation, Pelvis	0 -	1 (1++)	0 -	0 -
- Cysts	1 (1++)	0 -	0 -	0 -
Liver	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	7	5	7
- Chronic Infiltrates	0 -	1 (1+)	3 (3+)	1 (1+)
Mammary Gland	N=8	N=7	N=8	N=8
Number of animals within normal limits	8	7	8	8
Ovaries and Oviducts	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Pancreas	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	7	7
- Degenerative Changes	0 -	0 -	1 (1+)	1 (1++)
Pituitary Gland	N=1	N=8	N=8	N=8
Number of animals within normal limits	1	8	8	8
Spleen	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Uterus and Uterine Horns	N=8	N=8	N=8	N=8
Number of animals within normal limits	7	6	7	8
- Dilation	1 (1++)	2 (2++)	0 -	0 -
- Angiectasis	0 -	0 -	1 (1+++)	0 -

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Table 42: Histopathological lesions identified in unexposed F1 adult male rats (age 60-74 days), whose parents were exposed for 28 consecutive days.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Adrenal Glands	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Brain (Basal Ganglia; Cortex; Hippocampus)	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	8	8	8
Epididymis	N=8	N=8	N=8	N=8
Number of animals within normal limits	7	8	8	8
- Aspermia	1 (1+++)	0 -	0 -	0 -
Heart	N=8	N=8	N=8	N=8
Number of animals within normal limits	7	7	8	8
- Inflammation	0 -	1 (1+)	0 -	0 -
- Cardiomyopathy	1 (1+)	0 -	0 --	0 -
Kidneys	N=8	N=8	N=8	N=8
Number of animals within normal limits	3	5	5	6
- Chronic Infiltrates	2 (1+/1++)	0 -	0 -	1 (1+)
- Progressive Nephropathy	4 (2+/2++)	3 (3+)	0 -	1 (1++)
- Fibrosis	0 -	0 -	3 (3+)	0 -
- Cast, Proteinacious	0 -	0 -	1 (1+)	0 -
- Cysts	0 -	1 (1++)	0 -	0 -
Liver	N=8	N=8	N=8	N=8
Number of animals within normal limits	7	8	6	6
- Chronic Infiltrates	1 (1+)	0 -	2 (2+)	2 (2+)
Pancreas	N=8	N=7	N=8	N=7
Number of animals within normal limits	8	7	8	7
Pituitary Gland	N=5	N=8	N=8	N=8
Number of animals within normal limits	5	8	8	8
Spleen	N=8	N=8	N=8	N=8
Number of animals within normal limits	8	7	8	8
- Hyperplasia	0 -	1 (1+)	0 -	0 -
Testes	N=8	N=8	N=8	N=8
Number of animals within normal limits	7	8	8	8
- Degeneration, Seminiferous Tubule	1 (1+++)	0 -	0 -	0 -

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Table 43: Histopathological lesions identified in unexposed F1 adult male rats (age 60-74 days), whose mothers were exposed for 28 consecutive days and whose fathers were unexposed.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Adrenal Glands	N=8	N=8	N=7	N=8
Number of animals within normal limits	8	8	7	8
Brain (Basal Ganglia; Cortex; Hippocampus)	N=8	N=8	N=7	N=8
Number of animals within normal limits	8	8	7	8
Epididymis	N=8	N=8	N=7	N=8
Number of animals within normal limits	8	8	7	8
Heart	N=8	N=8	N=7	N=8
Number of animals within normal limits	8	8	7	8
Kidneys	N=8	N=8	N=7	N=8
Number of animals within normal limits	7	6	6	**2Ŧ**
- Chronic Infiltrates	1 (1+)	1 (1++)	1 (1+)	4 (2+/2++)
- Progressive Nephropathy	0 -	0 -	0 -	1 (1++)
- Cast, Proteinacious	0 -	0 -	0 -	1 (1+)
- Cysts	0 -	2 (2++)	0 -	2 (1+/1++)
Liver	N=8	N=8	N=7	N=8
Number of animals within normal limits	7	7	7	6
- Chronic Infiltrates	1 (1+)	1 (1+)	0 -	2 (2+)
Pancreas	N=8	N=8	N=7	N=7
Number of animals within normal limits	8	8	7	7
Pituitary Gland	N=1	N=8	N=7	N=8
Number of animals within normal limits	1	8	7	8
Spleen	N=8	N=8	N=7	N=8
Number of animals within normal limits	8	8	7	8
Testes	N=8	N=8	N=7	N=8
Number of animals within normal limits	8	8	7	8

Incidence (in parentheses) Severity: Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++

Ŧ Pearson Chi-square test indicated statistically significant differences between the dose groups and controls, which were validated by pair-wise comparisons using Fisher's Exact Test.

Table 44: Summary of neurobehavioral test results (± SE) in the P1 generation rats (parents) following a continuous 28-day exposure; n=32.

Subject Group	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Motor Activity (total activity time in seconds out of 30 minute test session)				
Male Parent	1530 ± 32	1429 ± 67	1422 ± 58	1402 ± 67
Female Parent	1563 ± 30	1523 ± 43	1439 ± 51	1499 ± 55
Watermaze Navigation (percentage of time spent in previous platform quadrant)				
Male Parent	33.6 ± 3.9	38.7 ± 2.3	33.4 ± 1.9	33.5 ± 2.1
Female Parent	33.2 ± 1.1	38.2 ± 2.8	32.2 ± 3.1	33.0 ± 4.3
Watermaze Navigation (number of crossings over previous platform location)				
Male Parent	3.0 ± 0.7	4.4 ± 0.6	4.4 ± 0.7	4.9 ± 0.5
Female Parent	3.3 ± 0.5	4.6 ± 0.7	3.7 ± 0.6	3.6 ± 0.9
Maternal Retrieval (seconds for dam to retrieve 3 PND2-3 pups removed from nest)				
Female Parent (parentally exposed pups)	50 ± 8	63 ± 7	48 ± 11	**137Ŧ ± 11**
Female Parent (maternally exposed pups)	43 ± 5	93 ± 31	58 ± 14	51 ± 11

Ŧ Two-way ANOVA indicated statistically significant differences (increased maternal retrieval time) between high dose group dams and controls, which were validated by pair-wise comparisons using Tukey-Kramer procedures.

Table 45: Summary of neurobehavioral tests results (± SE) in F1 infant rat pups, whose parent(s) were exposed to a continuous 28-day exposure prior to mating; n=128.

Subject Group	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Righting Reflex (seconds for pup at PND4-5 to rollover from supine to prone position)				
Male Pups (exposed parents)	9.9 ± 1.6	10.8 ± 2.1	9.0 ± 2.3	6.1 ± 1.2
Male Pups (maternal exposure only)	8.6 ± 1.6	5.6 ± 0.7	**11.2Ŧ ± 2.1**	6.6 ± 1.2
Female Pups (exposed parents)	11.5 ± 1.7	13.1 ± 2.1	12.2 ± 1.9	8.8 ± 1.5
Female Pups (maternal exposure only)	8.8 ± 1.1	12.8 ± 2.3	13.0 ± 2.0	8.2 ± 1.2
Separation Distress (number of ultrasonic distress vocalizations emitted per minute at PND 7-8 after pup separation from dam)				
Male Pups (exposed parents)	21.9 ± 4.3	16.6 ± 4.1	15.6 ± 4.1	19.6 ± 4.9
Male Pups (maternal exposure only)	39.7 ± 6.9	30.6 ± 4.9	33.0 ± 6.7	32.0 ± 6.5
Female Pups (exposed parents)	16.6 ± 3.0	20.0 ± 4.8	17.9 ± 4.5	**9.3Ψ ± 2.9**
Female Pups (maternal exposure only)	35.8 ± 8.9	31.7 ± 7.2	29.6 ± 4.3	**21.7Ψ ± 5.3**

Ŧ Two-way ANOVA indicated statistically significant differences (increased righting reflex time) between male pups of mid dose dams compared to male pups of high dose dams. Differences were validated by pair-wise comparison using Tukey-Kramer procedures.

Ψ Non-parametric one-way Kruskal-Wallis tests indicated statistically significant differences (decreased pup vocalizations) between the female pups from high dose group dams and sires in comparison to controls. Differences were validated by pair-wise comparisons using Conover-Inman procedures.

Table 46: Summary of neurobehavioral tests results (± SE) in F1 adult rat offspring, whose parent(s) were exposed to a continuous 28-day exposure prior to mating; n=32.

Subject Group	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Motor Activity (total activity time in seconds out of 30 minute test session)				
Male Offspring (exposed parents)	1487 ± 52	1356 ± 61	1294 ± 72	1286 ± 108
Male Offspring (maternal exposure only)	1429 ± 69	1395 ± 73	1392 ± 70	1339 ± 67
Female Offspring (exposed parents)	1479 ± 57	1432 ± 65	1339 ± 68	1366 ± 67
Female Offspring (maternal exposure only)	1525 ± 37	1456 ± 47	1482 ± 55	1447 ± 62
Watermaze Navigation (percentage of time spent in previous platform quadrant)				
Male Offspring (exposed parents)	29.4 ± 3.9	31.5 ± 4.6	27.0 ± 3.2	37.7 ± 3.8
Male Offspring (maternal exposure only)	36.1 ± 3.9	35.4 ± 6.2	35.9 ± 3.0	38.0 ± 4.4
Female Offspring (exposed parents)	34.3 ± 4.0	34.2 ± 2.2	30.4 ± 2.1	31.9 ± 2.8
Female Offspring (maternal exposure only)	35.7 ± 5.3	36.5 ± 3.6	36.5 ± 1.4	38.6 ± 4.0
Watermaze Navigation (number of crossings over previous platform location)				
Male Offspring (exposed parents)	2.9 ± 0.6	3.5 ± 1.0	2.0 ± 0.7	4.0 ± 0.7
Male Offspring (maternal exposure only)	3.9 ± 0.8	3.6 ± 1.3	4.1 ± 0.6	4.6 ± 1.0
Female Offspring (exposed parents)	4.7 ± 1.0	3.6 ± 0.9	3.1 ± 0.6	3.1 ± 0.6
Female Offspring (maternal exposure only)	3.3 ± 1.0	3.9 ± 0.7	2.6 ± 0.3	3.9 ± 0.7

FIGURE 1

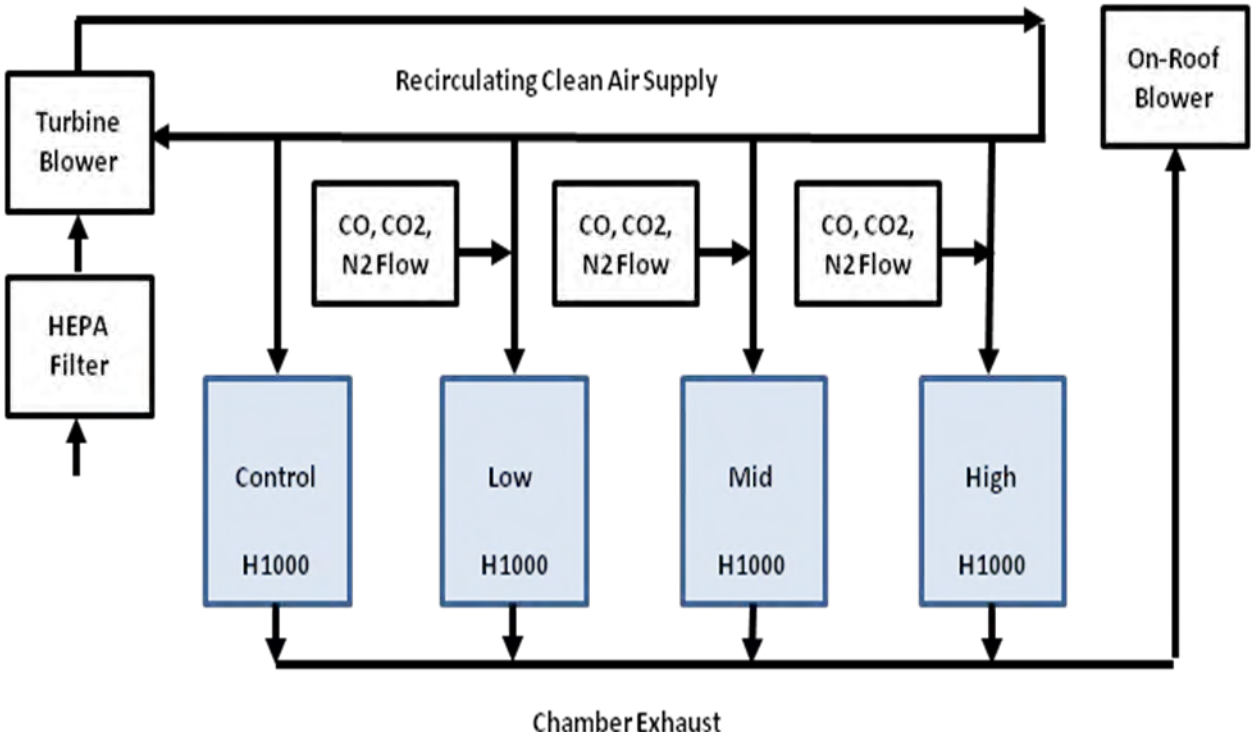

Figure 1. Inhalation exposures were conducted in H1000 inhalation chambers for a clean air control and three dose groups. Dilution air for each chamber was directed into the top of each chamber by a turbine blower. The turbine blower recirculated the excess air and pulled makeup air from the room through a HEPA filter. The test chemicals (CO, CO_2, N_2) were introduced into the dilution air for each chamber at flow rates to achieve the target concentrations for CO, CO_2, and O_2. Nitrogen was used as a test chemical to displace oxygen resulting in reduced oxygen concentrations. All air into the chamber was exhausted from the bottom of the chamber by a roof-mounted blower.

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6. AUTHORS
Daniel Hardt, Richard James, Chester Gut, Jr., Shawn McInturf, Michael Gargas

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Naval Medical Research Unit Dayton
2624 Q St., Bldg. 851, Area B
Wright-Patterson AFB, OH 45433-5707

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14. ABSTRACT

Neurological and reproductive performance were assessed in rats exposed 23 hours/day for 28-days to mixed gas atmospheres that represent submarine air quality standards for continuous exposure limits (CELs), as well as 24-hour and 1-hour emergency exposure limits (EELs). Exposure to 28 days of elevated concentrations of CO and CO2 under hypoxic conditions, representative of CELs and EELs, did not affect the ability of rats to reproduce and did not result in any significant developmental deficits in their offspring. Phase I and II of this study indicate that existing submarine air standards are health protective of male and female crew members. This report represents Phase II of the study to assess neurological and reproductive performance. Phase I was a range-finding study completed on 27 June 2011. The conclusive portion of this study will be Phase III, which will provide a 90-day, 2-generation, developmental and reproductive study using the same exposure criteria, which final report will be submitted in 2012.

15. SUBJECT TERMS
Inhalation, Carbon Monoxide, Carbon Dioxide, Hypoxia, Reproductive Toxicity

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